TRANSACTIONS

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No 5 Issued May 1943

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 18th May, 1944, at 3 p.m

THE PRESIDENT

SIR HAROLD SCOTT K.C.S.G M D , F.R.C.P , F R S E.
in the Chair

PAPER

BLACKWATER FEVER ANURIA

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I am afraid you will find this a scrappy paper   It is not possible to do justice to this brobdingnagian subject in anything less than book size so I can do no more than touch on a few points. I propose to discuss the alkaline treatment of blackwater fever and the lesions of the kidney found in the accompanying anuria, and I hope to show you that —

1   Intensive alkaline therapy has failed as a general treatment.

2. The hypothesis upon which it is based is not adequate to account for the renal failure which develops.

3   The kidney lesion seen in fatal cases of anuria is *not* peculiar to black water fever but is common to many other conditions in which renal failure occurs with or without haemoglobinuria.

It would be very interesting to go on to consider in detail some of the factors involved in the production of the renal lesion, but perhaps that is better left for the discussion which follows this paper

ALKALI THERAPY

EFFECT ON MORTALITY RATE AND DEVELOPMENT OF KIDNEY FAILURE.

Modern intensive alkali therapy aims primarily at producing an alkaline urine with the object of preventing the precipitation and deposition in the renal tubules of haemoglobin and its derivatives. Mechanical blockage of the uriniferous tubules is thus avoided and the kidney continues to function.

Since more than half the deaths in blackwater fever occur when the patient is, or has been anuric (STEPHENS, 1937) prevention of kidney blockage should appreciably lower the mortality rate of the disease.

This, however does not appear to be the case.

Intensive alkali treatment based on the mechanical blockage hypothesis was first tried by HANSCHELL in 1925

Today 19 years later after such therapy has been developed and brought into wide use, it can be shown that the average death-rate from blackwater fever allowing for geographical and yearly variation, *has not decreased*

Thus, in Table I are set out figures showing the mortality rates for the period prior to 1921

Compare these figures with those shown in Table II which gives the mortality rates recorded by a number of observers in cases which were given alkali treatment (by mouth, or intravenously or both)

Comparison of Tables I and II reveals the discouraging fact that the mortality rate since the introduction of alkali therapy has, if anything, increased.

It is more difficult to demonstrate the effect of alkali treatment on the appearance of renal failure in blackwater fever

TABLE I.

BLACKWATER FEVER.

MORTALITY RATE BEFORE INTRODUCTION OF ALKALINE TREATMENT

Authority	Year	No. of cases.	Deaths.	Mortality rate.		
STEPHENS (Summary)	1885–1922	4 991	952	Av 19% :	Varies from	0–60
THOMSON (S. Rhodesia)	1901–1910	516	131	Av 25% :	„	20–38
THOMSON (S. Rhodesia)	1911–1921	84..	118	Av 24% :	„	11–31

TABLE II

BLACKWATER FEVER.

MORTALITY RATE   CASES RECEIVING ALKALINE TREATMENT

Authority	Year	No. of cases.	Deaths.	Mortality Rate %
SHELLEY	1921	51	19	37
PATERSON	1933	35	12	33
FERNAN NUÑEZ	1936	52	—	about 20
SMITH & EVANS	1943	16	5	31
WEST AFRICA FORCE	1941–42	>150	—	>20

For instance, PATERSON (1933) reports in a small series of cases, that although he did not have encouraging results from the administration of alkali by mouth, ' early administration of intravenous sodium bicarbonate appears to have a preventive action on the development of urinary suppression." Similar treatment failed to affect the course of suppression once it had appeared.

Other authors have more gloomy reports to make and in West Africa, where alkali therapy was extensively used, renal failure was much the commonest fatal complication.

The final answer to this question must await the results if they are worth waiting for, of a long series of properly controlled cases of blackwater fever treated with and without alkali

Nevertheless, it has to be accepted that whether or no the alkali therapy has in general greatly affected the incidence of renal failure in the disease, it has certainly not succeeded in influencing the mortality

Since, as has been shown above alkali treatment has not reduced the general mortality rate of blackwater fever it becomes necessary to review the situation and consider —

(1.) The validity of the hypothesis upon which the therapy is based.

(2.) Whether the alkali treatment *per se* has a harmful effect.

(3.) Whether in certain individual cases such treatment may nevertheless benefit the patient

(1.) THE BASIC HYPOTHESIS.

The theoretical basis of intensive alkaline treatment has already been mentioned and need be recapitulated only briefly

Renal failure is brought about by mechanical blockage of the uriniferous tubules by haemoglobin or its derivatives (possibly acid haematin) Precipitation of these haem compounds takes place only in an acid medium and is

facilitated by the presence of an adequate concentration of sodium chloride. Consequently in the words of BAKER and DODDS (1925) —

> Since the factors leading to the precipitation of pigment are *acidity* and *salt concentration* of the urine any type of therapy tending to reduce these should prove of value The use of alkaline diuretics or transfusion of sodium bicarbonate as soon as possible should minimize the precipitation in the tubules."

If this mechanical blockage hypothesis is the correct explanation of what happens in the kidney in blackwater fever anuria, it is clear that certain conditions must obtain (*a*) The urine should be acid. (*b*) There should be adequate sodium chloride present in the urine. (*c*) Haemoglobin should be present. Let us consider each of these points in turn.

(*a*) *The urine should be acid* The evidence in the literature is very contradictory Statements of authors vary from when the urine contains much haemoglobin it is always alkaline " (PLEHN 1896)   normally acid, often hyperacid, sometimes neutral or alkaline especially at the decline of a crisis (GOUZIEN 1911) to ROSS s (1932) categoric comment that " when cases were excluded which were receiving alkali the reaction was invariably acid or neutral.

In West Africa the reactions of the urine passed immediately before and immediately after the onset of oliguria were recorded in a number of cases, all treated with alkali. From these it is clear that in both circumstances the urine may be alkaline as often as it is acid. This point is well shown in Table III

<div align="center">

TABLE III

WEST AFRICAN CASES (ANURIC OLIGURIC).
REACTIONS OF URINE.

A. *Effect of Alkaline Treatment.*

</div>

Reaction at onset of Blackwater Fever	Effect of Alkaline Treatment.				
	Urine				
	Stayed Acid.	became permanently Alkaline.		varied final urine being	
				Acid.	Alkaline
Acid twenty-five cases	5	7		6	5
Neutral two cases	All became alkaline.				
Alkaline five cases.	All remained alkaline.				

B. *Reaction of Urine in Relation to onset of Anuria or Oliguria*

	Reaction of Urine							
	(a) Immediately prior to onset of anuria/oliguria.				(b) First specimen after onset of anuria/oliguria.			
	Acid.	N	Alk.	Not known.	Acid	N	Alk.	Not known
I ANURIA No. of cases	8	3	8	10	16	—	10	1
II OLIGURIA No. of cases	1	—	4	—	1	—	4	—

It can thus be said that the first condition requisite to the blockage hypothesis is not fulfilled in more than about half the cases which go into anuria i.e. the urine is *not* always acid.

It is convenient to examine here the effect of alkaline administration on the reaction of the urine.

In normal healthy persons with sound kidneys the urine will become alkaline after the ingestion of small amounts of sodium bicarbonate (5–10 grammes sodium bicarbonate PALMER and VAN SLYKE, 1917.)

There is however, a mass of evidence to show that such is not the case in individuals who deviate from normal.

For instance, acid urines have often been reported in the presence of proved alkalosis of the tissues. Thus BROWN *et al* (1923) described a case of tetany with an acid urine, DIXON (1924) a case of tetany with a plasma CO_2 of 101 vols per cent. and a strongly acid urine GOLDWITZER MEIER (1924) a case with plasma CO_2 of 89 vols. per cent and an acid urine.

It has also been found that in the presence of previously damaged kidneys the urine may remain acid in spite of extensive alkali therapy (PALMER and VAN SLYKE, 1917.) This has been observed over and over again in the treatment of gastric and duodenal ulcer (ELLIS 1924 COOKE, 1932, etc.)

In blackwater fever similar failure of alkali treatment to alkalinize the urine has been frequently reported. For instance, PATERSON (1933) states that in his patients taking large doses of bicarbonate orally only two out of eight developed alkaline urine. This was also a common observation in West Africa, as will be seen by reference to Table III.

The conclusion is unavoidable that alkali treatment may fail to affect the reaction of the urine

(b) *There should be adequate sodium chloride present in the urine* (BAKER and

DODDS, 1925)   The sodium chloride concentration of the urine in blackwater fever has been studied by many authors   perhaps the most detailed account is that given by Ross. See Table IV

TABLE IV

SODIUM CHLORIDE CONTENT OF BLACKWATER FEVER URINE. (ROSS).

1 Early Urine :

NaCl%	0·05	0·1	0·	0·3	0·4	0·5	0·6	0·7	0·8
Number of cases	1	2	11	7	8	3*	1	0	1

2. Cases :

	1			2			3	
Day	Hb	NaCl%	Total (grammes)	Hb.	NaCl%	Total (grammes)	Hb.	NaCl%	Total (grammes)
1	+	0·16	1 26	+	0·17	1 23	+	0·11	1·6
2	+	0·16	8·47	—	0·28	0·84	+	0·09	* 2
3	—	0·42	6·95	+	0·06	0·48	—	0·05	1 78
4	—	0·5	9 06	+	0·06	0 86	—	0·06	1 37
5	—	0·44	8·6	+	0·06	0·80	+	0 21	3·92
6	—	0·54	10·49	—	0·06	0 24	—	0·81	10·32
7	+	—		+	0·04	0·41	—	0·84	11 12

Two cases immediately before suppression.

Examination of Ross s figures shows that in his cases, even immediately before the appearance of suppression, the NaCl concentration of the urine was much lower than 1 per cent. usually varying from 0·3 to 0·5 per cent., and that, in recovery the urinary NaCl concentration only slowly returned to normal limits. Similar low chloride concentrations are reported by other authors. For instance, Ross THOMSON et al. (1910) state that during black water fever and subsequently up to the 23rd day chlorides were markedly diminished "

GOUZIEN (1911) states that the fall of urinary NaCl is normally at a maximum about the 3rd to 4th day and recovers slowly In two cases it was 0·5 gramme per litre. WAKEMAN and MORRELL (1929) LAHILLE (1915) etc., have similarly noted the low excretion of NaCl. Very few figures for NaCl concentration of urine in blackwater fever oliguria were obtained in West Africa, but those available indicate a similar low concentration. It appears, therefore, that in blackwater fever the urinary NaCl concentration does not usually approach the figure at which it would facilitate precipitation of haem pigments.

(c) *Haemoglobin should be present* This, of course, is a crucial point. If

blockage is due to the deposition of precipitated haem compounds, then haemoglobin must be present as the source of the pigments. It is not possible to investigate this fully here owing to the tremendous dimensions of the literature on the subject. Only a brief summary can be given. In many cases of blackwater fever in which there is excessive destruction of red cells and intense haemoglobinuria (and the urine is acid) no anuria or oliguria develops. Again, in some cases of blackwater fever oliguria and anuria kidney failure develops after the haemoglobinuria has ceased (sometimes many hours later) and may fail to develop after recovery from oliguria, although haemoglobinuria may persist. Further, conditions other than blackwater fever, in which haemoglobinuria appears and may be excessive, only very seldom go on to anuria. This rarity of anuria in haemoglobinuric conditions other than blackwater fever has been commented on vigorously by GEORGOPOULOS (1933). Finally anuria and oliguria develop in conditions and in circumstances which exactly parallel the renal failure of blackwater fever but in which there is no haemoglobinuria.

Having noted that neither the acidity of the urine, the concentration of NaCl, nor the immediate presence of haemoglobin is always present in the blackwater fever patient who develops anuria it remains to consider whether mechanical blockage of the uriniferous tubules can in itself explain the failure of urinary flow. Our experience in West Africa was that the degree of blockage found in kidney tubules postmortem was insufficient to account for anuria. Thus, although some kidneys exhibited very considerable tubular obstruction, others from cases of complete anuria showed only minor degrees of blockage and extensive plugging was found in the kidneys of cases which had not passed into oliguria or anuria. (MAEGRAITH and FINDLAY, 1944.) These findings (a full account of which will eventually be available) are in agreement with those of other authors, for example, GEORGOPOULOS (1933) who consider that the blockage alone is not sufficiently extensive to account for complete anuria.

There are two other points which are difficult to explain on the mechanical blockage hypothesis. These are (i) the low concentration of urine passed during and after the oliguria and anuria, and (ii) the frequent absence of casts and debris in the urine passed during the post anuric phase and during recovery.

(i) It is difficult to fit in failure of the urine concentrating power of the kidney with simple mechanical blockage. If blockage were the primary factor it would be expected that what urine was passed, coming as it did, from unobstructed nephrons, would be normal in its constitution. In fact, this is not so. The urine, as we shall see later is dilute of low specific gravity and has a low salt and urea content. To account for this on the theory of blockage it would be necessary to show that the convoluted tubules have been so damaged by distension that they fail in their function and recover only slowly over a period of weeks. Histological evidence of such distension in the form of dilated capsular spaces and grossly dilated tubules is not a common finding in blackwater fever anuria.

(ii) With regard to the second point, although the urine passed in the immediate post anuric and recovery phases occasionally contains massive deposits, made up of tarry material (WAKEMAN 1929), it is often perfectly clear and relatively free from casts and other debris. Thus in the two West African cases which recovered from anuria, the urine on the 1st day of recovery in both was clear and contained, in one case "no casts" and, in the other some granular casts. In neither of these cases were large quantities of casts or debris passed at any subsequent time. Thus in Case 7 (Capt. KARANI), the reports on the urine read as follows: Day 8, "no urine" Day 9 "no casts" (2 oz.) Day 10 "no casts" (7 oz.) Day 11 clear (13} oz.) Day 12, a few granular casts (74 oz.) Day 13, "no casts" (47 oz.)

To summarize it may be said that the mechanical blockage hypothesis cannot account for the failure of urinary flow in blackwater fever since the urine is not always acid, the salt content of the urine is low the relation of anuria to haemoglobinuria is irregular the degree of blockage found post mortem is not always consistent with the degree of clinical renal failure, the urine passed during and immediately after anuria or oliguria is poorly concentrated and frequently contains no casts or debris.

Alkaline therapy is therefore based on a hypothesis that cannot explain the condition of anuria as observed in blackwater fever

(2.)   HAS ALKALI TREATMENT *per se* A HARMFUL EFFECT ?

There is abundant evidence in the literature to show that impairment of renal function can be brought about by intensive administration of alkali, such, for example as that employed in the treatment of gastric and duodenal ulcer Such damage to kidney function is particularly well shown in patients with previous renal impairment and can also be observed (McCANCE and WIDDOWSON 1937) in patients who are rendered dehydrated and chloride deficient as the result of persistent vomiting In renal insufficiency arising from such alkali dosage the syndrome is very similar to that seen in kidney failure in blackwater fever although anuria is not a common complication. There is the same nitrogen retention and lowering of blood chloride there is the same failure of urinary urea concentration the same lesions are met in the kidneys (McLETCHIE, 1943)

These observations have an obvious bearing on the alkaline treatment of blackwater fever in which there is often vomiting dehydration in the early stages, and some salt deficiency This is especially so when we consider that the dosages of alkali employed in blackwater fever are of an order similar to those used in the treatment of ulcer and that in blackwater fever we are dealing with kidneys which are probably damaged and may become anuric.

That the dosages of alkali employed in the treatment of blackwater fever are very high can be verified by looking into almost any modern textbook. Physicians are repeatedly told to "push alkali until the urine becomes alkaline"

in spite of the fact that in a high proportion of cases the urine never in fact *will* become alkaline. (See above.)

Thus Low and FAIRLEY (1941) in *Price's Textbook of Medicine* Fluids are pushed per os and should contain sufficient sodium bicarbonate and potassium citrate to alkalinize the urine and so lessen the clogging of the renal tubules 150 c.c. of 3 per cent. solution of sodium citrate (4 5 grammes) is sufficient to render the urine temporarily alkaline alternatively 8 grammes of potassium citrate per os has the same effect. To keep the pH of the urine on the alkaline side of pH 7·0, 35 grammes of potassium citrate should be given in the 24 hours. When the urine has a brownish tint the urine requires alkalinization.

SMITH and EVANS (1943) are even more enthusiastic, advising intensive alkaline therapy enough alkali being given to produce Trousseau's sign. Elsewhere these authors state that in treating malaria the *blood* should be made alkaline as a routine the assumption that in cases which develop clinical tetany *the pH actually would approach 8*

Alkaline treatment of cases in West Africa was developed along lines such as those laid down by Low and FAIRLEY and varied from a few grammes of sodium bicarbonate to as much as 80 to 100 grammes in a single day Intravenous sodium bicarbonate and sodium lactate were also widely used.

The patient in blackwater fever is usually vomiting and sweating profusely and consequently losing both fluid and salt the former is frequently replaced (often to excess) but the latter may not be, so that the patient may become relatively salt deficient. (This is also indicated by the low plasma chloride usually reported in the disease) Consequently since it has been shown that even small amounts of alkali can upset kidney function in dehydration and salt deficiency (COOKE, 1932) it is clear that the massive doses of alkali (40 to 100 grammes in a day) administered to the blackwater fever patient may have a depressing effect on his renal function.

It can thus be said that there is a very strong possibility that the alkaline treatment itself may do considerable harm to the patient by impairing his kidney function.

(3) DO SOME CASES BENEFIT FROM ALKALINE TREATMENT?

It is difficult to deny that some of the cases reported in the literature did benefit amazingly from alkaline treatment.

The beer-drinking patient of HANSCHELL (1925) is a case in point. Here it does seem that the alkali may have been the deciding factor in restoring the renal activity

If then, some cases do benefit from alkaline treatment in spite of what has been said above how can the alkali work?

It is possible in the first place that some patients may be saved from acidosis and accompanying impairment of renal function (BERGER and BINGER,

1935 KOEHLER, 1927), by the timely administration of alkali. The occasional occurrence of acidosis in blackwater fever has been established and the effect of alkali therapy on such acidosis has been described by FAIRLEY and BROMFIELD (1934)

In addition to assisting the acidotic patient, alkalis must in limited quantities also exert their physiological effects as diuretics and may thus serve a useful purpose.

Summing up the position of intensive alkaline treatment, I would say :—

1 The treatment used as a general therapy has not been successful it is based on a hypothesis which does not completely fit the facts.

2 The excessive use of alkali is probably dangerous in blackwater fever where kidney function is likely to be impaired and salt may be deficient.

3 Alkaline treatment may be useful for individual cases where acidosis is threatened or has developed, or where a mild saline diuretic would be of value.

If alkaline therapy is to be used it must be controlled and should not be pushed simply because the urine does not become acid It could be controlled by measurement of plasma CO_2 (e.g., by Conway's method 1939), where laboratories are available (an upper arbitrary limit being set, say at 80 vols. per cent.) or by the reaction of the urine after moderate administration of alkali (say 15 grammes). If the alkali reserve rises fast and the urine reaction does not change, then continuation of alkaline treatment is not indicated.

THE SYNDROME OF ANURIA IN BLACKWATER FEVER.

It has been shown above that the mechanical blockage theory is not adequate to explain the facts of renal failure in blackwater fever It would, therefore, be interesting to devote some time to the consideration of alternative hypotheses (FOY ALTMANN et al., 1943) This is not possible here.

It is necessary now however to define as clearly as possible what the renal condition is in blackwater fever and perhaps to suggest a few lines along which the future research may develop

The patient. The condition of the patient in anuric or oliguric blackwater fever is familiar to many of you. Let it suffice to say that the general condition throughout the acute phase resembles shock '' and is frequently in the later stages accompanied by the characteristic motor hyperactivity and vomiting of uraemia. The blood pressure varies considerably from patient to patient, but it is usually low in the early stages of anuria (and immediately prior to its development) and subsequently rises as renal failure develops frequently there is a collapse towards the end, marked by a fall in systolic, and a more exaggerated drop in diastolic, pressure. (See Figs. 1 and 3.)

The blood. Changes in blood content of various kinds develop before, during and for some time after the onset of anuria.

Blood urea rises steadily throughout the anuric phase It may be elevated and rise slowly for some days before urinary flow fails, but once urinary flow is grossly reduced the blood urea rises very rapidly
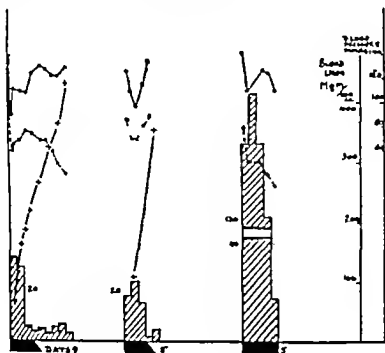
FIG. 1
(Cases 24 23 29)
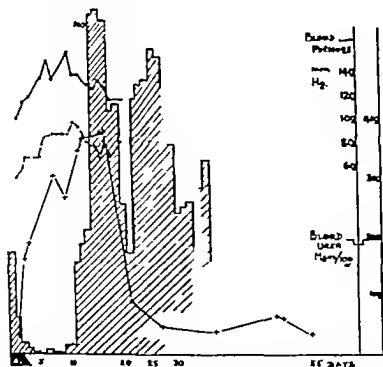
FIG. 2.
(Case 36.)

In fatal cases the blood urea may reach fantastic levels, but in recovery the blood urea curve traces a very characteristic path which is well illustrated in Cases 36 and 7 (See Figs. 2 and 3)

The blood urea may go on rising steadily for several days after the re-establishment of urinary flow even during the post-anuric polyuric period or it may remain level for a few days. After some days, however it begins to fall and reaches normal limits 2 to 3 weeks after recovery of the urinary flow   (See Figs. 2 and 3.)
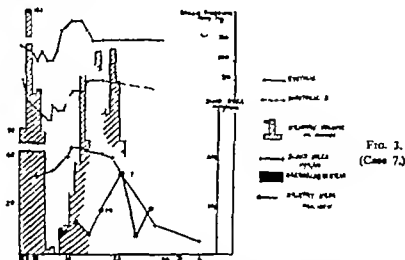
FIG. 3.
(Case 7.)

A mild degree of nitrogen retention is found in most blackwater fever cases, even in the absence of failure of kidney function. FAIRLEY and BROMFIELD (1934), for instance, report blood ureas of as high as 79 in mild cases, WHITMORE and ROE (1929), go as far as to say   in all our cases there is evidence of N retention even those which progress favourably

Other changes   While the blood urea is rising there normally develops a pronounced fall in plasma Cl which, in recovery returns slowly to normal, and in fatal cases progresses rapidly to low levels. Blood phosphates do not alter significantly

The Urine   During anuria or oliguria and in the recovery stages, the urine shows signs of being poorly concentrated. The specific gravity is low (Ross, THOMSON *et al.*, 1910 KRAUSE, 1904 WERALKO 1926 etc.). Urea concentration is poor 0·5 per cent. or less (WAKEMAN 1929 WAKEMAN and MORRELL, 1929 OWEN and MURGATROYD 1928 etc.)   The sodium chloride concentration is low (GEORGOPOULOS, 1933 ROSS 1932 WAKEMAN and MORRELL, 1929 etc.).

All these points were noted in the West African cases in which measurements were made. For example a specimen of urine examined on the 3rd day of anuria in one case showed a concentration of 0·38 per cent, NaCl (7½ oz. urine passed in 24 hours)   Urinary urea was also low and took 2 to 3 weeks to return to normal even after the post-anuric diuresis had finished. (See Fig. 3.)

THE KIDNEY LESION

The pattern of the lesion is of very great importance —

Distribution of Blood

Subcapsular plexus This is not mentioned by most authorities, but in the kidneys from West African cases it was generally found that the blood vessels were dilated and filled with blood. In some cases this region was congested.

Cortex The vessels of the cortex are not engorged or congested, except in irregular minute areas. Most authorities refer to the cortex as being 'normal' or even 'anaemic' (the latter possibly in relation to the state of the vessels in the medulla). The vessels of the glomeruli are usually empty or nearly so. Only occasionally are congested glomerular tufts described. The lack of congestion of the cortical vessels and the relative emptiness of the glomerular tufts was one of the regular features of the West African cases.

Medulla and Sinus All observers are agreed that the medullary vessels are frankly congested. The vessels are distended and packed with blood cells and in places appear to have broken into adjacent tubules, which are themselves filled with blood cells. Sometimes this medullary congestion is enormous and widespread sometimes it is patchy. But it is always present.

The Parenchyma

Tubules The tubular epithelium is affected mainly in the ascending loop of Henle and in the distal convoluted tubules especially in the macula densa region. The epithelium elsewhere is affected to some extent in an irregular manner but the damage to the distal convoluted tubule seems invariably present. The changes in the epithelium have been described so often that repetition is unnecessary beyond pointing out that the lesion affects chiefly the cytoplasm and to a less degree the nuclei and basement membrane so that the lumina are filled to a varying degree with cellular debris. The cells are often greatly reduced in depth so that many of the tubules appear dilated. Some real dilatation is sometimes seen, but is not a constant feature of the condition. The 'plugs' of haemoglobin or its derivatives when they occur are to be found mostly in the distal convoluted tubules, the ascending loops of Henle and in the collecting tubules. They are uncommon in the proximal tubules. As has been mentioned above their numbers vary considerably and do not disclose any obvious relation to the urinary flow.

Glomeruli

As was noted above, these are frequently empty of blood and hardly ever congested. The capsular space does not often show any great enlargement suggestive of ' back pressure " but this is very difficult to gauge as

Foy et al. (1944) point out, in sections fixed in formalin. The space may contain some coagulated debris but, as often as not, is quite clear.

Let us summarize what has just been said.

In the kidney failure of blackwater fever we have a state in which —

1   There is a clinical condition resembling " shock " and sometimes passing into uraemia.

2.   Diminution of urinary flow occurs.   This usually develops suddenly and possibly indicates rapidly developing failure of glomerular filtration.   It is accompanied by progressive nitrogen retention in the blood, and later in the post anuric phase, by the passage of large quantities of dilute urine   Recovery of nitrogen excretion lags far behind the recovery of urine flow

3   The changes in the kidneys are congestion of the medullary vessels relative " anaemia " of cortex, including glomeruli   degenerative changes in the tubular epithelium, especially in the region of the ascending loop of Henle and the distal convoluted tubules   the presence of haemoglobin products or haemoglobin-stained casts in the tubules.

The above combination of oliguria or anuria occurring in the course of an acute illness and followed, in cases that survive, by a post-anuric stage of impaired renal function revealed by nitrogen retention and the passage of copious dilute urine (HAVARD) occurs not only in blackwater fever but in many conditions, such as incompatible transfusion (DE NAVASQUEZ, 1940)   crush syndrome (BYWATERS and BEALL, 1941   BYWATERS and DIBLE, 1942, etc.)   alkalosis (McLETCHIE, 1943 etc.)   septic abortion (BRATTON, 1942)   concealed accidental haemorrhage (YOUNG, 1942)   cholera (ROGERS, 1921   CHATTERJEE 1941 etc.)   yellow fever (MAEGRAITH, 1942)   sulphonamide haemoglinuria (FOY et al., 1944)   mercurial and bismuth poisoning (FISHBERG 1939)

This syndrome has been exactly reproduced in rabbits by the injection of lithium monourate (DUNN and POLSON, 1926) and in rats by administering large doses of acid sodium phosphate (McFARLANE, 1941)

For the sake of convenience, my colleague, Sgn. Lieut. HAVARD and I suggest that this state might be called " the tubulo-vascular renal syndrome "

The development of such a syndrome cannot at the moment be explained. There are, however many interesting lines upon which profitable work could be based.

For instance, the syndrome is common to many conditions   It can be produced by very simple substances, such as phosphate   a very restricted portion of the kidney is mainly involved.   It may be possible, therefore, that the determining factor in the production of the syndrome in the various conditions in which it is found is either the same or acting in the same way

Since the lesion affects mainly the distal convoluted tubule it is useful to consider in what way this part of the kidney differs from the rest.

Structure   The cells of these tubules are smaller than those of the proximal tubules.   They are columnar and their nuclei are packed closer together

This difference in structure may indicate a difference in function, as has been suggested by DUNN *et al* (1941) who consider (from experiments on urate and phosphate kidney damage) that this level of the nephron is mainly concerned with the excretion of acids.

Blood supply The distal convoluted tubules of the mammalian kidney approximate themselves very intimately to the afferent vessels of the glomerulus with which they are associated. It is not known for certain whether this is a ' return of the nephron to its mother glomerulus, ' but it is likely that the main blood supply of the distal convoluted tubule consists of blood which has not traversed the glomerulus but comes direct from either the interlobular artery, its non-glomerular descending branches or the vessels arising from the glomerular afferent arteriole. The blood supply therefore probably differs from that of the proximal tubules which are partly supplied by the glomerular *efferents* and partly by the general cortical plexus, including branches from the subcapsular plexus.

It is conceivable, therefore that the distal tubular function may be interfered with in the tubulo-vascular syndrome either by a direct alteration of function, possibly arising from saturation with acid or basic radicles (DUNN *et al* 1941) or an indirect alteration of function arising from anaemia and redistribution of blood flow through the kidney

Diminution of blood flow to the distal tubules would result in interference with the function of the epithelium by either lack of oxygen and consequent changes of metabolism, or by collapse of the vessels into the tubules or vice versa, with consequent reabsorption of urine into the circulation (WINTON 1937 BYWATERS and BEALL, 1941 etc.)

There is some evidence that such circulatory changes can take place. For example the experiments of McCANCE and WIDDOWSON on salt deficient individuals led them to the conclusion that there were changes produced in glomerular flow and filtration. Again, the experiments of CORCORAN and PAGE (1943) on hypotension and transfusion in dogs have led those authors to state   It seems likely that renal flow during hypotension is distributed *irregularly* through the renal vascular bed, being greatest in sites of less resistance.

Work on these lines, using diodrast and inulin clearances as indicators might, I believe, assist in solving this problem. Examination of the peripheral blood flow and the tone of arteriolar and minute vessels of the skin, carried out on the lines of DI PALMA (1941-42-43) or by the simple process of studying the responses of skin vessels to stroking is also desirable. The few cases examined on these lines in West Africa indicated very clearly that changes in peripheral flow and vascular tone do occur in blackwater fever

To recapitulate —
1 Alkali therapy has failed as a general treatment.
2. The hypothesis upon which it is based is not adequate.

3   The kidney lesion may be coupled with the clinical condition and allied with that seen in other diseases to form a common syndrome which we have labelled for argument a sake the *tubulo-vascular renal syndrome"*

4   I have suggested a few lines of future investigation.

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Discussion

Dr A C Howard asked if Colonel Maegrath could give any figures on the mortality in blackwater fever from anuria as opposed to that from other causes. He then described a case he had seen in West Africa where death had occurred from anaemia, in which anuria had occurred for 3 days the last specimen of urine passed before the onset of anuria was completely black but the urine obtained by catheter just before death (only about 6 oz.) was clear with no sediment at all. The postmortem examination of the kidneys showed them to be comparatively normal with no evidence of tubular blockage He suggested that the anuria was due to anaemia.

Dr A C de B Helme The "Experts" seem backward ! There is one slight observation I might make on a clinical case which occurred in East Africa An Indian boy had had malaria for many years He was 17 years of age. He came in with blackwater fever and had been taking quinine We tried him on atebrin hoping that the fever would drop it did but when we increased the dosage that increased the production of blackwater We tried him on very small doses of quinine again increasing and producing the black water We tried him on alkalis that did the same. The only treatment that did any good was masterly inactivity combined with a certain amount of glucose flavoured with lime juice in water

The point I wish to make is that whatever we did including the exhibition of alkalis, seemed to increase the severity of the blackwater

Brigadier J A Sinton congratulated Colonel Maegrath upon his valuable contribution to the literature of the treatment of blackwater fever

Colonel Maegrath appeared to have based his observations on the idea that the anuria of blackwater fever was due mainly to a precipitation of blood pigment in the renal tubules with a mechanical blockage and that the alkaline

treatment of this condition was started in an attempt to prevent such precipitation or to resolve it when formed. This idea is based on the work of BAKER and DODDS (1974), but the alkaline treatment is of a much earlier origin. Two old treatments of blackwater fever—HEARSAY's and STERNBERG's—may both be called alkaline to some extent. MACGILCHRIST (1913)* considered that haemoglobinuria in malarial patients was associated with a condition of "acidosis," and recommended alkaline treatment. When the quinine and alkali treatment of malaria was started it was also suggested that large doses of alkali given with quinine medication might help to prevent the onset of haemoglobinuria in cases predisposed to this condition.

The work of Colonel MAEGRAITH goes to show that such mechanical obstruction is not the whole, nor possibly even most, of the story in the anuria of blackwater fever and most observers will agree. One cannot however for this reason alone, condemn the alkaline treatment for there are other possible factors in the causation of this condition in which such treatment may be beneficial.

A similar anuria may occur in the terminal stages of fatal cases of cholera, where there is no question of mechanical blockage by blood pigment. In this disease, in common with blackwater fever there are seen dehydration salt depletion, acidosis and toxaemia, and sometimes also a low blood pressure.

While an alkaline urine probably diminishes the tendency to the precipitation of haemin in the renal tubules, the good effects which follow the exhibition of alkaline treatment in the anuria of cholera (ROGERS), suggest that such treatment may act in other beneficial ways.

Some of these ways are possible actions on —

(a) "*Acidosis*" Alkaline treatment should benefit this and so diminish its effects, both local and general.

(b) *Damage to renal epithelium* The high urea concentration reported in the blood, combined with the low urinary one, appears to indicate considerable damage to the excretory powers of the kidney Whether this damage, so well seen in microscopical sections, is due to "acidosis," "toxaemia" or anoxia due in low blood pressure local or general, is a wider subject There is however considerable evidence to suggest that alkalis have some protective action against the effects of various poisons and toxins on the renal epithelium (see summary by SINTON and LAL, 1924)† If this damage can be prevented or diminished it should have an important effect upon the occurrence and duration of anuria, by helping excretion and possibly also by diminishing local falls in intrarenal blood pressure with resultant anoxia.

(c) *Local intrarenal blood pressure* There is some suggestion that the anuria may be the result of low general blood pressure. In cholera at least, this does not always appear to be the case My experience in the latter

* MACGILCHRIST (1913) *Indian J med. Res* 1 p. 160.

† SINTON AND LAL (1924) *Ibid.,* 12 p 47

disease has been that even when the general blood pressure was raised and maintained at a high level by intravenous injections this frequently failed to re-establish urinary secretion. If low blood pressure be the main cause of such failures it has possibly a local distribution inside the kidney.

There is some evidence to support such a view. Colonel MAEGRAITH has told us that in his experience the glomerular capillaries are usually empty while the more proximal arterioles are often congested. This suggests a local obstruction. ROGERS (1922)* has reported that with the kidneys of patients dying of cholera three or four times as much pressure was required to force saline solution through the blood vessels than in the case of similar organs from patients dying of other diseases. These observations suggest a local obstruction to blood flow and so diminished blood pressure in the area beyond the obstruction. In this case the low blood pressure would probably be in the region of the glomerular capillaries.

What suggestions may be made as to the causation of this obstruction? The arterioles of the kidney are small and run in very close association with the excreting tubules. May it not be possible that in an organ with such an inelastic capsule as the kidney has the swelling of the tubular epithelium seen in blackwater fever may result in mechanical pressure on these arterioles causing a reduced blood supply to the glomeruli and diminished blood pressure there? Such an obstruction would result in diminished excretion as well as anoxia and further cellular damage. If as noted above alkaline treatment has some protective action on the renal epithelium this might also help to diminish the causes of this mechanical obstruction to blood flow.

The alkaline treatment of blackwater fever has been reported upon favourably by many clinical observers over a number of years. In view of the great variations in the mortality recorded in blackwater fever the scientific value of the alkaline treatment is difficult to assess but this clinical evidence cannot be disregarded completely. Before the alkaline treatment can be condemned entirely much more evidence is needed and Colonel MAEGRAITH's work should do much to stimulate research in this direction. A controlled series of trials by the alternative case method should be started with cases treated with and without alkalis.

Sir Philip Manson-Bahr One of the sole advantages of this war is that it has brought fresh minds to bear on age-old problems. Blackwater is a case in point. For some time past it has become evident that the older ideas on mechanical blockage of the urinary tubules do not afford a wholly satisfactory explanation of anuria. Recently study of this intricate subject has been aided and abetted by similar happenings in such totally dissimilar states as crush injury and favism. It is therefore clear in view of what Colonel MAEGRAITH has told us that we must marshal our facts all over again. Is it not possible,

* ROGERS (1922) in Byam and Archibald's *Practice of Medicine in the Tropics* Vol. II p. 1096

in regard to his theory of the relation of the distribution of blood vessels to the convoluted tubules that haemolysis may originate, as PLEHN originally held, from masses of subtertian parasites in this particular area. The remainder of the blackwater syndrome may well be explained on an allergic basis analogous to that of the haemoglobinuria of favism. The biochemical changes which have been described are secondary phenomena, but that injury to the renal convoluted tubules constitutes a primary factor in anuria can hardly be held in doubt. Colonel MAEGRAITH did not say what was the result of blood transfusion in these anuric cases. It probably had no great effect upon the restoration of urinary flow but it had always, with some show of reason, been regarded as a life-saving measure and in his own experience of the treatment of blackwater he had on several occasions every reason to believe that his patients owed their lives to it.

Dr H S STANNUS With regard to the alkali treatment of blackwater fever—if my memory serves me, it was introduced about 1904 as an adaptation of that in use in yellow fever i.e. more than 20 years before the date mentioned by Colonel MAEGRAITH.

My chief interest, however has been in Colonel MAEGRAITH's reference to what he has called " the tubulo-vascular syndrome " found not only in the anuria of blackwater fever but in other conditions as well. (I would have suggested the term " capillary nephron syndrome.")

Recently in connection with another subject I pointed out the enormous importance of the capillary system as the system most immediately concerned with furnishing to the interstitial spaces all the essentials for preserving at a constant the " milieu intérieur " of CLAUDE BERNARD or internal environment of every cell in the body without which normal metabolism of the cells of a tissue is interfered with. Further I suggested that though nothing is known on the subject, it would be fair to presume that the cells of the capillary endothelium themselves must require these same essentials for their metabolic needs, including oxygen, sugar various metabolites vitamins, etc., and that any failure of supply must fall first on the tissue forming the capillary endothelium and cause a derangement of function of these vessels, for which I used the term " capillary dysergia." In blackwater fever the sudden and often extreme haemolysis presumably induces a marked lowering of the oxygen-carrying capacity of the blood with the production of anoxia This will cause a loss of tone of the capillaries, dilatation and diminished flow possibly stagnation the functions of the endothelium will be depressed and secondarily the " milieu intérieur " disturbed and the normal metabolism of tissue cells interfered with. The number and arrangement of capillaries in a tissue are such as to meet its normal requirement with considerable variation in different tissues. In the kidney in which different parts of the nephron serve widely differing functions, this is well seen It is only necessary to call to mind the difference between the thick cubical cells of the convoluted

tubules and their renals and the very thin epithelium lining Bowman's capsule which with the basement membrane and endothelium of the capillary together only measure 0·001 mm. in thickness. This suggests the reason why the same cause may lead to failure in different functions of the kidney.

It is along these lines that I think further research will eventually solve the problem of anuria in blackwater fever. The same failure of capillary function doubtless causes the heart failure in that disease.

Dr C. O. Chesterman. The speaker has made it clear that alkalinity of the urine precipitate does not make much difference to anuria. Has he any idea as to the relation between the reaction of the urine and the initial haemolysis? Is it not more likely to occur in acid urine than in alkaline? Has he any information to confirm the report from East Africa of the efficacy of large doses of phenobarbitone in arresting haemoglobinuria?

Colonel Maegraith (in reply). I think the first point raised concerning anaemia fits in with what Dr Stannus was saying that anaemia as such and apart from changes in circulation would act equally well on the functions of the tubule cell and upon the capillary itself. It is possible in supreme degrees of anaemia, for example in pernicious anaemia (FISHBERG 1939[*]) that the anaemia may be the exciting factor but I do not think it is the fundamental process involved.

With regard to the use of glucose and " masterly inactivity " I look upon " masterly inactivity " as another way of saying control of alkali treatment." One of the two cases which recovered was treated with very large doses of glucose intravenously and during the period of exhibition of glucose the alkali treatment was stopped. The patient recovered and it was thought that recovery was due to glucose but I think it was due, at any rate partly to limitation of the alkali treatment. In answer to Brigadier Sinton I must apologize for not putting alkaline treatment further back in the history of this disease but it became a question of what to leave out rather than what to say. I am well aware that alkali was used for a long while before 1925 but I was referring in my paper to massive and intravenous treatment which I think was not used before 1925. With regard to the suggestion that the circulation may be interfered with by the changes in the epithelial cells producing pressure in the tubules and thus exerting pressure back through the lumen membrane against the capillary I think the point is that you have to get a very considerable increase in intrarenal tension before you get much change in kidney function (WINTON 1937[†]). I think such rise of intrarenal tension would be indicated in the histological picture by dilatation of the tubules concerned. such dilatation is not very frequently observed. The epithelial cells when damaged are usually shed into the lumen.

[*] FISHBERG, A. M. (1939). *Hypertension and Nephritis* London Baillière, Tindall & Cox.
[†] WINTON F. R. (1937). *Physiol. Rev.*, 17 408.

I cannot off hand state the results of blood transfusion. I am afraid one thing that may influence the whole of the argument about alkalosis is that a common factor in many of these West African cases was blood transfusion. We considered blood transfusion to have its chief value in restoring to the circulation the red cells capable of carrying oxygen to the tissues needing them, and we refrained from blood transfusion until the blood cell count fell to a limit which we considered dangerous from the point of view of failure to carry oxygen to the tissues.

I have never seen accumulation of parasites in the kidney vessels such as suggested by Sir Philip Manson-Bahr.

An important point has been raised by Dr Stannus concerning anoxia and the effect of anoxia on the minute blood vessels. There is a certain amount of evidence on this point in the blackwater fever cases in West Africa. I refrained from mentioning this except very briefly in my paper because of time but if you undertake the simple process of examining the skin reactions of patients suffering from blackwater fever—if you rub a pencil or a thermometer case along the arm, you get a very good indication of what is actually happening to the minute vessels of the skin. I think Dr Goddard (who treated one of these cases and who is here today) will bear me out when I say that in the few cases in which this was tried we were able to show that during the acute phases of blackwater fever there was a definite indication of atonia of the small vessels which probably originated from damage to those vessels resulting from lack of oxygen supply. The atonia was evidenced principally by difficulty in obtaining a white line. In the healthy arm, where circulation is normal, if you examine the skin along the path where the pencil is rubbed you will see a white line which results from constriction of the small vessels beneath the path of the instrument. This constriction depends upon two things the power of the small vessels to constrict and the ability of the small vessels, once constricted, to remain constricted.

If the venous pressure is raised in the normal arm the white line will vanish when the pressure reaches about 100 mm Hg. The counteracting venous pressure capable of causing the disappearance of the white line was much lower than normal in the few cases of blackwater fever we examined. This means that in these cases the small vessels were more weakly contractile and were constricted less powerfully than in health in other words they were atonic.

Experiments on renal clearances of inulin and diodrast could, I think, reasonably be performed in humans to investigate renal blood flow not only in blackwater fever patients but in healthy subjects. In this way a great deal of valuable information would be obtained about intrarenal circulation

in the disease and under laboratory controlled conditions Such experiments are now in progress at Oxford

With regard to any relation between the reaction of the urine and the degree of initial haemolysis my impression is that there is none. I have had no experience with phenobarbitone.

DEMONSTRATION OF MICROSCOPE SLIDES

Exo-erythrocytic Forms of *Plasmodium gallinaceum* in Tissue Culture.

By

F HAWKING D.M. (Oxford) D.T.M.

Besides the well-known cycles of development in the invertebrate host (mosquito) and vertebrate host (chicken) this malaria parasite also grows in macrophages and other endothelial cells these stages discovered by JAMES and TATE, are the "exo-erythrocytic" forms. Their relation to the forms which occur in the erythrocytes is unknown possibly they constitute a connecting link between the sporozoites injected into the vertebrate host by the mosquito and the trophozoites eventually found in the red blood corpuscles They have not been demonstrated in human malaria.

Cultivation *in vitro* of the endo-erythrocytic forms of malaria parasites is not yet possible, but the exo-erythrocytic forms can easily be grown by the standard techniques of tissue culture Macrophages are cultured from the blood or spleen of chicks inoculated 8 days previously with sporozoites The medium consists of 30 per cent serum and 70 per cent Tyrode's solution containing a trace of embryo extract 0.05 per cent phenol red and 5 units penicillin per c.c. to assist in the maintenance of sterility The cultures are conveniently grown on small pieces of coverslip attached by plasma clot to the floor of a Carrel flask.* When required the piece of coverslip can be taken out of the flask and the culture is fixed and stained *in situ* Multiplication of the parasites occurs vigorously in these cultures as was shown by the slides and photographs demonstrated Many of the macrophages contain multiple infections eighteen or more parasites being found in one cell In some cultures, large clusters of elongated merozoites are found apparently derived from the breaking up of ripe schizonts Up to date, parasites have been demonstrated after 60 days in these cultures by microscopical examination and after 90 days by the infection of chickens A preliminary account of this work was published in the *Lancet* †

COMMUNICATIONS

ACUTE HEPATITIS WITHOUT JAUNDICE IN WEST AFRICA

BY

R. W. BROOKFIELD M.D., PH.D (LIV.) M.R.C.P (LOND.) LT.-COL. R.A.M.C.*

This note is concerned with a series of cases of hepatitis encountered in Africans of military age during a year's work in the medical division of a West African General Hospital. It is not claimed that all the cases have the same aetiology but certain common features enable them to be grouped together for the purposes of discussion. All have been characterized by an acute febrile illness of considerable severity and enlargement of the liver accompanied by extreme tenderness. Fixation of the diaphragm and inflammatory disturbance in the right lung have been frequent. Jaundice and bilinuria have been absent and there has been no manifest disturbance of liver function. Apart from slight enlargement in some cases most likely to be due to antecedent malaria there has been no evidence of concomitant disease of the spleen. There has been no clear evidence of gastro-intestinal disease. Vomiting has been uncommon. Alcoholism did not seem to be a factor nor could the hepatitis be ascribed to any drug. Efforts to find an infective agent, whether bacterial protozoal or metazoal have been unsuccessful and the cases have been roughly classified for descriptive purposes according to their apparent response to various forms of treatment.

The close affinity of this form of hepatitis with the symptomatology of amoebic hepatitis will be immediately recognized and it is proposed first to indicate the extent to which amoebiasis has been met with in the hospital

and then to describe those cases whose clinical features and therapeutic responses approximate most closely to those of amoebic hepatitis.

AMOEBIC DYSENTERY AND TROPICAL ABSCESS

The incidence of amoebic dysentery has been fairly high. Of the 2,158 patients admitted to the Medical Division during the year there were 156 cases of dysentery of various types. Forty nine were proved cases of amoebiasis and a small number of others, showing a non bacillary type of exudate but without the presence of entamoebae or their cysts, only resolved after the exhibition of emetine. Despite this incidence there were only two cases of obvious hepatic amoebiasis with abscess formation.

The first case was that of a patient, Cpl M S., with chronic dysentery and proved fatal. It was chiefly noteworthy in that the liver was both small and non-tender. Protozoa were not recovered from the wall of the abscess cavity but were present in sections of the bowel wall.

The other case, that of Sgt. G E. had many points in common with the group to be discussed later. He gave no history of dysentery or diarrhoea. The onset was sudden with fever followed next day by pain and tenderness in the right hypochondrium. There was no jaundice or splenic enlargement. Impairment of note and diminished movement and breath sounds at the right base suggested early pneumonia, but after 33 grammes of sulphapyridine his pain was unrelieved, there was still remittent pyrexia and the lower border of his liver was found to be well below the right costal margin. The leucocyte count was 29 000 per c mm with 80 per cent. polymorphs. X ray confirmed the presence of a large pleural effusion which on tapping proved to be a straw coloured opalescent fluid containing lymphocytes. It was sterile. At a later exploration pus with a brownish tinge was obtained. This was likewise sterile and when evacuation by aspiration was carried out it was found to be subdiaphragmatic, while above the diaphragm there still persisted the serous effusion which had been tapped on the first occasion. Injections of emetine hydrochloride were begun and were followed up by a combined course of emetine bismuth iodide orally and chiniofon rectally. The temperature fell to normal on the 7th day of treatment and the liver quickly returned to its normal size.

Repeated examination of the stools failed to demonstrate any amoebae or cysts in this case, nor were entamoebae found in the pus, but its characters, the course of the illness and the response to emetine seem to admit of no doubt as to the accuracy of the diagnosis of amoebic abscess of the liver.

RESOLUTION OF HEPATITIS

HEPATITIS RESOLVING AFTER EMETINE.

An account will now be given of several cases closely resembling the one of amoebic abscess just described, in that continued pyrexia, tender hepatic enlargement and signs in the right lung all resolved on the exhibition of emetine. There was no history of dysentery save in one patient, who admitted having had the disease 2 years previously. In no case was *Entamoeba histolytica* recovered from the stools. Evidence of suppuration was lacking in these cases, of which the following are examples —

Pte O A. was admitted complaining of abdominal pain and feverishness which had been present for 5 days. Tonacity was marked. He was very tender over the liver which

was enlarged three fingers breadths below the right costal margin. Breathing was distressed The breath sounds were diminished at the right base and rhonchi were audible A white cell count revealed a leucocytosis of 28,900 Entamoebae or their cysts were not found in the stools nor was there any history of diarrhoea Emetine hydrochloride was given hypodermically and in 5 days the temperature was normal. The physical signs resolved rapidly and he was ready for discharge to duty after 29 days in hospital

Pte H. Y. had been suffering from pain in the chest and abdomen for 11 days before his admission to hospital. There was tenderness and rigidity in the right hypochondrium making palpation difficult, but his liver appeared to be enlarged. There were no chest symptoms and signs and the radiograph of his chest merely showed fixation of the right diaphragm. Examination of the blood film stools and urinary deposit showed no abnormality The blood culture agglutination reactions Kahn and Ide tests all gave negative results. The white cell count was 10,300 with 54 per cent polymorphs 36 per cent lymphocytes, 3 per cent. eosinophils 7 per cent. monocytes Pyrexia to 101 F continued unabated but fell after the exhibition of emetine which was begun on the 18th day of his illness The local tenderness was relieved and his temperature finally settled on the 25th day He returned to his unit 39 days after the onset

A similar clinical picture was presented by nine other cases. All had a large tender liver In many tenderness was for a time exquisite and the resultant rigidity made definition of the size of the liver difficult. Enlargement was progressive until emetine was given, though it did not exceed three fingers breadths below the right costal margin When palpation was possible the surface was found to be smooth and the consistency approximately normal The temperature was irregular, tending to the remittent type with a maximum rise to about 103° F The degree of toxicity in some was such that they were placed on the dangerously ill list. Besides the cases already described chest signs were present in six others and indeed were such a prominent feature that five of these patients were thought to be suffering from lobar pneumonia and were given courses of sulphapyridine One of them had pleural friction and a small effusion was shown in the radiograph and in another an X ray examination showed incomplete consolidation of the right lower lobe In a third the symptoms and signs of pneumonia completely dominated the picture for the first 6 days of the illness. Cough with sputum, dyspnoea and physical signs of consolidation of the right upper lobe, confirmed radiologically were all present. Sulphapyridine failed to bring down the temperature and on the 7th day there was marked tenderness in the right hypochondrium and the liver was enlarged downwards one and a half fingers breadths Administration of emetine was begun After 2 days the tenderness had disappeared and the temperature fell It was not always practicable to make an X ray examination owing to the severity of the illness In four cases the diaphragm was raised and fixed, in three no abnormality was observed. Only in the cases already quoted was there radiological evidence of disease of the pleura or lung parenchyma The white cell count generally showed a moderate leucocytosis or gave a high normal result The figure given by Pte O. A. (28,900) was much in excess of any of the others. The usual agglutination reactions, including the Weil Felix were carried out in three cases with negative results. Blood culture was undertaken in three cases and in each

instance was sterile. The Kahn or Ide test, done in three cases, was weakly positive in one. Two patients were sigmoidoscoped in the convalescent phase. The mucous membrane was normal. Drugs other than emetine proved ineffective Quinine was given in practically every case without result and the pain and pyrexia were not mitigated by sulphapyridine in the patients to whom it was given. Emetine was strikingly effective in 1 to 3 days in most of the cases. In three however emetine had been given for 7 days before the temperature fell to normal In two of these, including Pte. H. 1., the pyrexia had already been present for 17 days and in the other for 9 days. In the light of other cases, where recovery occurred without the exhibition of emetine, the specificity of the drug in these three patients is not clearly demonstrated

The dosage of emetine hydrochloride employed was 1 grain daily for 12 doses In some instances this was followed up by a course of emetine bismuth iodide, with or without enemata of chiniofon, with the idea of ensuring the elimination of protozoa from the bowel In two cases emetine bismuth iodide was relied on exclusively 3 grains daily were given for 8 days and the temperature fell after 2 and 6 days respectively So far as is known, no patient experienced any further trouble

In the cases of hepatitis just described there was a close connection between the pyrexia and the liver involvement which had their onset, ran their course, and ultimately resolved *pari passu* In two further cases the temperature fell to normal before emetine was given. Hepatic enlargement and tenderness, however persisted and only subsided when emetine was administered.

Pte. A. S. became feverish, began to cough and complained of pain in the right shoulder and right lower chest. The pain was very severe after his admission to hospital on the 7th day of his illness There were signs of bronchitis. His pyrexia, which reached 10⁴·8° F., continued for a further 4 days and subsided a day after the administration of quinine had been begun Malarial parasites were not found. The tender enlargement of the liver did not recede though his temperature remained normal. Resolution, however occurred following a course of emetine hydrochloride begun on the 16th day of his illness. The liver had returned to apparent normality by the 20th day and he was discharged to light duty 4 days later This man had an attack of diarrhoea a week before his illness began, but there was no diarrhoea while he was in hospital. His stools were examined four times during this period atypical cysts were found on two occasions but were not regarded as those of *E hystolytica*.

The other patient, Pte U B experienced pain in the left hypochondrium on the 6th day of a febrile illness. Enlargement of the liver followed and persisted after the fall of temperature which occurred on the 37th day Quinine had already proved ineffective but following a course of emetine the liver returned to its normal size and resolution was apparently complete by the 40th day

On the whole the administration of emetine appears to have been the determining factor in the recovery of all these cases. In some however opportunity was not given to discover whether recovery would have taken place spontaneously apart from emetine because of the urgency of the symptoms

It was unjustifiable to withhold what might be a life saving drug and emetine was therefore given without delay

HEPATITIS RESOLVING WITHOUT EMETINE.

That spontaneous recovery can take place is suggested by the following case —

Pte T. E. became suddenly ill with pain in the right hypochondrium and pyrexia. There was marked tenderness and rigidity over the liver which was slightly enlarged on admission. This enlargement had increased by the 6th day to one extending three fingers breadths below the right costal margin. There was no cough and except for weakness of the breath sounds at the right base no chest signs while screening on the 10th day showed no abnormality. The pyrexia lasted 3 days only reaching a maximum of 103 F and medication was withheld. Malarial parasites were not found. White cells numbered 10,800. The Kahn test was negative. On the 14th day the liver was only just palpable. The patient was seen again 39 days after the onset of his illness when the lower border of his liver could not be felt.

Whether the aetiology was the same in this case as in those previously dealt with is an open question

HEPATITIS RESOLVING AFTER QUININE.

Two patients—the onset of whose illnesses was in every way comparable to that of those previously described except for the absence of chest signs—appeared to recover after the administration of quinine

Pte D. O. was admitted with pyrexia and epigastric pain and tenderness. The accompanying rigidity made palpation of the liver edge impossible but this organ was enlarged to percussion. Examination of the blood smear and the stools yielded negative results. Pyrexia running up to 102.4 F and pain persisted. Quinine was not given till the 9th day of the illness. The temperature fell next day rose again in tertian fashion the following day and then subsided completely as did also the pain and hepatic enlargement. He was discharged to duty on the 19th day from the onset of his illness.

In Pte A. L.'s case quinine was given at an earlier stage. Pyrexia and a very tender enlarged liver were present from the onset. No malarial parasites were found and examination of the stools gave a negative result. Quinine was begun on the 3rd day of the illness and the temperature came down to normal and the liver tenderness subsided on the 25th day. Vague abdominal pain continued till the 11th day. He returned to duty 17 days after the onset of his illness.

It is impossible to be sure that it was the quinine which led to resolution in these cases, but the time relation between the exhibition of the drug and the fall in the temperature is suggestive. Quinine was tried in many of the cases already dealt with without success

ACUTE HEPATITIS ASSOCIATED WITH SYPHILIS

It has been observed that in the secondary stage of syphilis in the African the pyrexia, which is generally mild and transient in the European, may be severe and prolonged necessitating the careful elimination of other causes

of continued fever such as typhoid. Two cases showing prolonged pyrexia of this type also had enlarged and tender livers.

Pte. A. B. while under treatment for gonorrhoea and a penile sore was suddenly seized with epigastric pain and tenderness and became feverish. There was severe hiccough. The symptoms abated somewhat, but not the fever and 2 weeks later he developed a pustular rash, at first taken for chickenpox. This, however did not resolve for many weeks and low pyrexia followed the usual sharp fever. Epigastric pain and some hepatic enlargement persisted. Examination of the stools gave a negative result and the leucocyte count was 8,200. The Idc test, initially negative became positive. Attention being principally focused on the liver a course of twelve injections of emetine hydrochloride was given with relief of both pain and fever. A month later, however there was further pain and another ten injections were given with apparent relief. Antisyphilitic treatment had been given throughout and the skin lesions had responded well. The response of the hepatitis to this medication was neither immediate nor dramatic, but after 3 months treatment recovery appeared to be complete and he was discharged to duty

While a recent syphilitic infection and an attack of hepatitis of some other aetiology may have coincided in this patient it seems more reasonable to suppose that responsibility for the total illness rested on the *Treponema pallidum* rather than on two independent infective process acting simultaneously

The second patient, Pte A. O., had been ill for a month before admission with what was thought to be pneumonia. The response to sulphapyridine was unsatisfactory His pyrexia continued and pain in the right hypochondrium was followed by progressive enlargement of the liver A course of emetine had no appreciable effect. Apart from an initial leucocytosis of 13 500 all investigations were negative except the Kahn test, which was positive Treatment with neoarsphenamine and sulpharsphenamine, though not very well tolerated at first, was persevered with and after about 10 weeks fever his general condition slowly improved. The pain and tenderness over his liver were relieved, but enlargement of the organ extending to within an inch of the umbilicus persisted. H was finally discharged to his home

In this case the diagnosis of syphilitic disease of the liver rests largely on the positive Kahn test and is perhaps not beyond dispute. Arsenical treatment seemed, however, to play a decisive role in arresting the progress of a hepatitis which had already inflicted serious damage on the liver and it is therefore concluded that the whole condition was in fact syphilitic.

REFRACTORY CASES.

Of the three cases now to be described two had ultimately to be invalided for chronic pain in the right hypochondrium while one was submitted to an exploratory laparotomy and was able to return to duty There was no response to emetine.

Pte D D had suffered from attacks of right hypochondriac pain on three occasions since 1940. He had an attack of dysentery lasting for a month during the East African campaign. He was admitted with fever and pain and tenderness in the right hypochondrium. The liver could not be felt. There was slight cough and diminished air entry at the right base. The radiograph showed the right diaphragm to be raised. Cysts were present in the stools but none typical of *E histolytica* were seen. Tenderness over the liver was persistent and he also had pain to the right shoulder. The pyrexia lasted for a month during the middle of this period a course of twelve injections of emetine hydrochloride was given without benefit. He had improved sufficiently after 6 weeks in hospital

to return to his unit, but was admitted 5 days later with recrudescence of his pain, for which he ultimately had to be invalided

Pte A. M. was admitted with a history of right hypochondriac pain for 1 month On admission his temperature was only 99 F but his liver was enlarged to two and a half fingers breadths below the right costal margin. X ray showed a bulge at the inner end of the right diaphragm. The cholecystogram was normal Emetine gave no relief and after 2 months in hospital without improvement he was invalided

The case of Pte A. E. was similar in onset to the acuter type of case already described. Pain in the right side of the abdomen and neck was accompanied by exquisite tenderness over the liver which rapidly enlarged to four fingers breadths below the right costal margin. There was irregular pyrexia running up to 104 F Investigations were negative Emetine was started on the second day after admission but the pyrexia continued for a further 12 days and when it subsided the pain still persisted and the condition of the liver was unchanged. The cholecystogram was negative but cholecystitis was suspected and in default of improvement laparotomy was undertaken There were numerous adhesions between the liver and the abdominal wall. The gall bladder was small and thickened It contained clear bile There were no stones. The liver was hard smooth and whitish. The glands in the hilum were enlarged, but not hard. Biopsy was deemed to be inadvisable The patient made a good recovery from his operation and there was little complaint of pain or tenderness afterwards He spent 4 months in hospital in all

PATHOLOGICAL CONSIDERATIONS

If the one patient with amoebic abscess referred to at the beginning of this paper be excepted there have been no deaths in this series of cases No help in determining aetiology has therefore been forthcoming from morbid anatomy Aspiration biopsy was not undertaken owing to the fear of haemorrhage from a congested liver The records of the twenty nine autopsies performed in the hospital during the same year have been studied with special reference to evidence of previous liver disease Adhesions to the abdominal wall pointing to old perihepatitis were present in six cases two of which also had a symptomless multilobular cirrhosis. Major W A Young informs me that microscopic sections of the liver of other cases failed to reveal any evidence of antecedent liver disease It is an open question as to whether hepatitis of the type under consideration may lead on to cirrhosis which is not uncommon among Africans and is by no means always due to alcohol. It is perhaps worthy of mention that only one typical case of cirrhosis of the liver with ascites was met with during the year He was a young subject and there was no evidence as to the aetiology

DISCUSSION

In seeking to establish the cause of the hepatitis in the cases that have been described it is not necessary to assume that the syndrome of pyrexia associated with a tender enlarged liver must be due to the same pathogenic agent in every instance Case histories have been given which suggest that malaria and syphilis may on occasion produce a hepatitis of this type There seems to be little doubt that those patients who responded so promptly to medication with emetine were suffering from amoebiasis Amoebic dysentery

was endemic in the areas from which the patients came and the symptomatology approximated closely to that of a developing amoebic abscess. At first sight the absence of a history of dysentery in all but one case and the failure of technicians well trained in the recognition of amoebae and their cysts to find these organisms in the stools might seem to be an obstacle to the diagnosis. However it frequently happens that in subjects with tropical abscess entamoebae can be identified in the wall of the abscess cavity despite the fact that there has been no antecedent dysentery and that repeated examinations of the stools by experienced workers have failed to detect either amoebae or evidence of colitis.

One feature which it is a little difficult to fit into the clinico-pathological picture of a disease primarily affecting the portal area, is the type of pulmonary lesion met with. Basal pleurisy with associated pneumonic change is common in hepatic amoebiasis, either as a result of direct involvement, or secondary to reflex fixation of the diaphragm, but it tends to occur late. Pulmonary signs are likely to be encountered where an abscess has already formed and is pointing towards the diaphragm. It is an unexpected finding therefore, that in the present series changes in the lung have antedated the first indication of inflammation of the liver by a number of days and have not been limited to the base.

In ascribing the greater number of the cases to amoebic infection it is realized that too much stress should not be placed on the therapeutic test and that amoebiasis is not the only disease which responds in a specific manner to emetine. This drug is credited with a hardly less striking effect in schistosomiasis, fascioliasis and paragonimiasis. The possibility of such infestation has not been lost sight of in the present series. Schistosomiasis has been common and seventy-two cases of the urinary form of the disease were treated during the year. Rectal schistosomiasis, which is prone to cause enlargement of the liver as in Egyptian splenomegaly has been less common — only eight cases were met with. However in none of the hepatitis subjects were there any features to suggest schistosomiasis and the splenomegaly which is prominently associated with hepatic schistosomiasis has been absent. Thus there is no indication that other diseases, favourably influenced by emetine, have played a part. The residual cases where there was no specific response to any form of medication still require explanation, but they have proved a baffling problem. In addition to the possible causes already referred to, attention has been directed to further types of infection or infestation. There was no evidence of infestation by any other helminths likely to affect the liver and it is noteworthy that eosinophilia was not a feature. Kala-azar seemed to be a possibility but this disease is almost unknown in West Africa and the characteristic leucopoenia was not present. Paratyphoid, tick fever, trypanosomiasis and undulant fever were considered only to be dismissed.

It is plain, therefore, that the problem of aetiology is a difficult one. No completely satisfactory explanation covering all the cases can be adduced and it is not unlikely that some of them including on the one hand those which show no response to emetine and yet fail to suppurate and on the other those that recover spontaneously, are due to some infective agent or agents which have not hitherto been identified.

Summary

1. The clinical features of twenty-one cases of acute hepatitis unaccompanied by jaundice have been analysed.

2. The symptomatologies of the cases have been similar conforming closely to that of amoebic hepatitis and being in many cases favourably influenced by emetine.

3. Pneumonic features have preceded the onset in certain cases.

4. Unequivocal evidence of antecedent amoebic dysentery has not been obtained nor has *Entamoeba histolytica* or its cysts been isolated.

5. Certain cases have shown no response to emetine or have recovered without it. Cases responding to quinine and to arsenicals are described. None have gone on to suppuration.

6. The question of aetiology is discussed.

INFECTIVE HEPATITIS IN PALESTINE

BY

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The so-called catarrhal jaundice has attracted the attention of the medical profession in Palestine since the British occupation in 1918 Nothing is known about its occurrence prior to that date but since then the disease has appeared in minor epidemics especially amongst the Jewish immigrants of the post war period That the disease is not new in Palestine is evident from the experience of medical practitioners especially paediatricians, all over the country The disease is common in children both Arabs and Jews but rare amongst the adult local population In children it is very mild and of short duration and therefore it escapes the notice of the hospital medical officers. It seems to confer an immunity for life hence its conspicuous absence in the adult population

The endemicity of the disease in Palestine was recognized by Leikowitz (1936) and Jossem (1940) described its periodic occurrence amongst the students of an agricultural school. Laboratory investigation including animal inocula tion performed both at private and official institutions failed to elucidate the causal organism though it served to rule out the possibility of spirochaetal infection.

The Jewish immigration to Palestine and the increase in the British Police Force during the last few years resulted in the appearance of many cases amongst the adult population through the increase in the number of susceptible individuals In 1938 our attention was called to the relatively large number of cases of "jaundice" amongst the British and Jewish members of the Police Force as compared with the number of cases amongst the Arab members Consequent investigation revealed that out of seventy-four policemen treated for "jaundice" at the Government Hospital Haifa during the period 1939 to 1941 there were forty British, twenty-eight Jewish and only six Arab

cases No reason could be found for this discrepancy All three sections of the Police Force lived side by side especially in the rural areas, and no relevant factors in the diet or habits of the three sections could account for the different incidence of the disease amongst them. Another striking observation was the fact that though " jaundice " was fairly frequent amongst the police guarding the Athlit Jail Labour Camp, the inmates of the camp, who are all Arab adult individuals seemed to enjoy complete immunity from the disease.
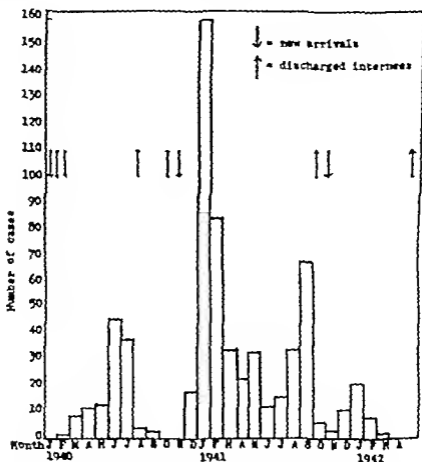
GRAPH I

In 1940 a clearance camp was opened at Athlit for the reception of the Jewish refugees arriving from Europe. Shortly after their arrival, an epidemic of infective hepatitis broke out in the camp and for the next two years successive epidemics made their appearance as new waves of refugees arrived (see Graph I). This gave us a unique opportunity for the study of various

aspects of the disease As the refugees stayed in the camp for relatively long periods of time, we were able to observe the disease from its commencement until after the cure A fair proportion of the cases were observed in hospital while the rest were treated as out patients

The camp was situated in the coastal plain 20 km south of Haifa, between the Athlit Jail Labour Camp and the Athlit Village and about 2 km from the nearest inhabited locality As both the Jail Labour Camp and the Athlit Village had in the past supplied our hospital with many cases of " jaundice " the appearance of the epidemic in the clearance camp was not unexpected There were altogether three major epidemics involving 633 persons during the two years of the existence of the camp Though it is believed that the first epidemic was started within the precincts of the camp the second epidemic was definitely proved to have been imported from Haifa Town A detailed study of the first two epidemics was published by KLIGLER, BTESIL and KOCIT (1943).

The discharged internees from the camp carried the infection with them to places where it was not known and minor epidemics occurred in several localities during the years 1941 and 1942 Dr J M SHAPIRO has actually been able to trace at least one epidemic in a far away settlement following the arrival of discharged internees from Athlit (personal communication) LEFKOWITZ (1943) noted the sharp rise of infective hepatitis amongst the members of the Workers Sick Fund towards the end of 1941 As this institution caters for the majority of the people in the Jewish settlements it is not improbable that this rise was due at least in part to the spread of the infection from Athlit

CAMERON (1941) reported on the incidence of infective hepatitis in the British Armies stationed in Palestine and concluded that the source of the infection was the native population

GEOGRAPHICAL DISTRIBUTION

Infective hepatitis is prevalent both in the rural and urban areas along the Mediterranean coast where the population is more dense

In the urban areas the cases are more or less sporadic though several cases are at times reported from schools and kindergartens In the rural areas the disease appears in periodic epidemics usually following the arrival of new settlers Certain localities are known to be " jaundice areas " and the appearance of cases in these localities can usually be predicted.

The prevalence of the disease in certain localities and its absence from others is unexplained There is a possibility of an animal reservoir but this has never been proved The disease tends to acquire epidemic proportions in places like schools camps prisons etc where people are crowded together

INCIDENCE.

Seasonal Incidence

The incidence of infective hepatitis is definitely influenced by climatic conditions. Though sporadic cases occur all through the year epidemic outbursts tend to occur in the autumn and early winter and sometimes towards the end of the spring. Graph II shows the distribution of the cases at Athlit and Graph III shows the same for the Haifa Hospital. The true seasonal

GRAPH 2   GRAPH 3
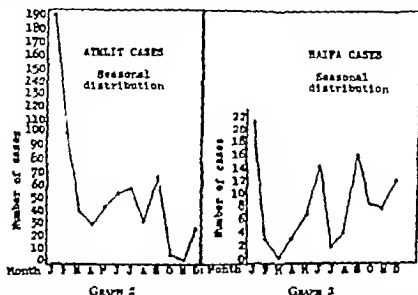
incidence is probably best inferred from the Haifa cases, as in the Athlit camp the number of cases depended on the camp population and on the arrival of susceptible individuals.

Sex Incidence

The sex distribution was as follows —

		Athlit Cases	Haifa Cases
Males		362	103
Females		271	14
	Total	633	117

Though our graphs show a predominance of male patients, this need not necessarily mean that females are less susceptible to the disease. The morbidity rate in Athlit was the same for males and females, while in Haifa, if we deduct the number of policemen from the total number of cases, the figures for the civilian population would be   Males 29 females 14   Thus

difference may be explained by other reasons such as occupation overcrowding etc.

Age Distribution

The majority of the cases occur in the age groups 16 to 30 years but older persons are also affected. The incidence of the disease in childhood and infancy is difficult to estimate since infective hepatitis assumes a mild form in children and is often undiagnosed. Still a fair impression may be obtained from the study of localized epidemics. At Athlit during the second epidemic (January 1941) the total population in the camp consisted of 913 men 636 women and 123 children aged 1 to 15 years. The incidence of hepatitis during that epidemic was as follows. Men 202 cases or 22.1 per cent. Women 103 cases or 22.0 per cent, and Children forty-eight cases or 42.3 per cent. Shapiro in a study of the incidence of jaundice in various parts of the country places the incidence in children at about 30 per cent while that in adults at only 2 to 4 per cent. (Unpublished *Report on the incidence of jaundice in the Jewish Settlements and villages* 1940 Dr J M Shapiro Department of Health.)

Race Incidence and Morbidity

All the cases from Athlit occurred amongst Jews coming from Germany Austria Czechoslovakia and Poland. In this connection it is interesting to note that there were 500 Bulgarian Jewish refugees in the camp but no cases of hepatitis occurred amongst them. The Haifa cases were distributed as follows. Arabs nineteen Jews, forty British fifty-three Others three.

The morbidity rate of the various sections of the population as evident from the number of hospital admissions during the period 1939 to 1941 was as follows —

	Total Admissions	Cases of Hepatitis	Percentage
Jews	2 856	40	1.4
Arabs	9 007	19	0.21
British	1 870	53	2.8

The figure of 1.4 per cent for the Jewish population is an underestimate as our hospital caters for only a fraction of the Jewish patients there being other institutions for the treatment of Jews. On the other hand the Government Hospital at Haifa is the only one which caters for the Arabs and the large majority of the British patients in the district. Lerkowrtz found that for the period 1141 to 31.3.43 the morbidity rate of infective hepatitis amongst the members (Jewish) of the Workers Sick Fund was 2.88 per cent.

Given favourable circumstances for the spread of the disease, namely overcrowding climatic conditions and susceptible individuals the morbidity

becomes much higher than the figures quoted above Thus, KLIGLER et al found that though during the first epidemic at Athlit the morbidity rate was 5 per cent., during the second epidemic not less than 24 per cent. of people in the camp developed the disease During both epidemics the total camp population was the same and there was no difference in the living conditions or food The only difference was that the first epidemic occurred in the spring when people spent a considerable part of their time outdoors while the second epidemic occurred in the autumn and early winter when people were more apt to crowd indoors.

INCUBATION PERIOD AND PERIOD OF INFECTIVITY

In the Athlit cases the shortest incubation was found to be 24 days Most cases developed the disease 40 to 45 days after their arrival at the camp but in some cases people developed the disease 2 or even 3 months after their arrival CASTEROS gives the incubation period as being 32 days while LEFKOWITZ, in a local epidemic found the incubation period to be from 21 to 31 days with an average of 28 days A Danish sailor treated at the Government Hospital Haifa, developed the disease 21 days after the arrival of his ship to Haifa harbour there being no history of exposure before

The period of infectivity is still unknown CASTEROS states that "No opinion can be given of the duration of infectivity though it obviously covers part of the incubation period pre-icteric phase and part at least of the icteric. The following case appears to show that the disease is infective during the incubation period

A police sergeant was transferred to Haifa from Athlit, and he developed jaundice 4 weeks after his transfer A few days later a police constable living in the same billet developed jaundice There had been no cases of jaundice from that billet prior to the arrival of the sergeant

There is some evidence to show that the disease may be infective after apparent clinical cure

A man working in a colony where infective hepatitis is endemic, developed the disease and was treated in the colony until the icterus had disappeared and the patient considered cured He was then given leave which was spent with his wife a school teacher in Jerusalem, living in a school where there had been no cases of hepatitis Four weeks after the man's arrival at the school his wife developed fever followed by jaundice (Dr J M SHAPIRO, personal communication.)

CLINICAL PICTURE

There are usually no prodromal symptoms, the disease having a sudden onset Occasionally some gastro-intestinal symptoms like anorexia epigastric heaviness diarrhoea or vomiting may precede the onset of the fever The clinical course of the disease may be divided into the following stages (1) Initial fever (2) Intermediate period (3) Hepatotoxic stage, and (4) Jaundice

(1) Initial fever The onset is marked by general malaise headaches, a

slight chill and a sudden rise of temperature which may reach 39 to 40° C within a few hours. Pains in the joints may be complained of the conjunctiva becomes congested there may be vomiting and a general feeling of apathy During the winter months upper respiratory catarrh may be present.

Physical examination reveals nothing of importance. In a small per centage of the cases the spleen may be palpable. The fever lasts from 1 to 4 days and finally falls gradually or by crisis. The diagnosis at this stage is usually sand fly fever or influenza.

The entity of this initial fever has been a subject of controversy. CAMERO who noted this type of onset in a certain number of his patients tends to consider this initial rise of temperature as being some other condition (sand fly fever etc) which lowers the body resistance and so permits the conversion of an earlier acquired latent infection to an active disease. We are unable to agree with this view for various reasons. The seasonal incidence of sand fly fever is not the same as that of infective hepatitis. The same is true of influenza. Furthermore if this initial fever is only an intercurrent infection then infective hepatitis should have been found more commonly following some of the other infections existent in the camp. Thus there were three typhoid epidemics in the camp involving altogether about 100 cases. In only one case was typhoid followed by infective hepatitis. In this case the typhoid fever lasted 28 days and on the 46th day the patient developed infective hepatitis showing all four stages of the disease including the initial fever (see Chart 1). As the patient was all this time in hospital and had no contact
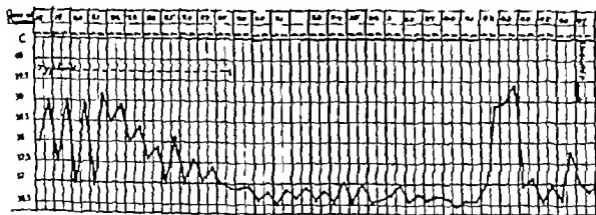
CHART 1 — Infective hepatitis following typhoid fever

with cases of infective hepatitis, we presume that the infection had been con tracted prior to her admission. As a matter of fact though typhoid fever is an endemic disease in Palestine and hundreds of cases are admitted yearly to our hospital we are unable to recollect any case followed by infective hepatitis. The same is true of malaria of which there were minor epidemics in the camp. Sand-fly fever was very common in the camp and all our cases

condition   The jaundice may be so deep and the liver so tender that laparatomies have been performed in some cases with the mistaken diagnosis of obstruction of the bile passages   In the milder cases icterus may be the only sign of the disease the patient feeling otherwise quite normal
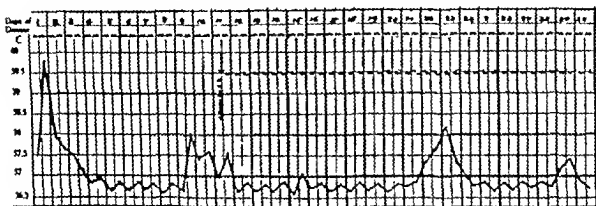
CHART 3 —Course of infective hepatitis

Chart 3 shows a typical case of the disease demonstrating all the various stages described above.

Clinical Varieties

The typical picture described above was observed in only 23 per cent of the cases   In the rest of the cases we may meet with the following clinical varieties —

1   In 34 per cent of the cases there was no initial fever the disease being ushered in by the hepatotoxic stage   In these cases the jaundice appeared on the 2nd or 3rd day of the fever   In some cases the fever may be so slight as to pass unnoticed by both doctor and patient but it is doubtful whether it is altogether absent.

2   The intermediate period was absent in 10 per cent of the cases Here jaundice appeared after 8 or 10 days of fever   In some cases there was a slight remission on the 4th or 5th day (Chart 4) In three cases the fever continued for many days after the appearance of jaundice (typhoidal type) and in one case the fever lasted 3 months   The diagnostic difficulties presented by such cases are obvious

3   In 33 per cent of the cases there was a definite hepatitis but no obvious jaundice   The existence of these cases of hepatitis sine ictero " was suspected by various observers   Careful observation at Athlit proved the existence of such a condition   In some cases there may be short periods of slight icterus and at times a person suffering from hepatitis without jaundice for several weeks or months may suddenly develop marked icterus

4 Finally the disease may consist of only the initial fever the patient showing no signs of hepatic involvement. The existence of these cases is of course difficult to prove at present as rises of temperature for 2 or 3 days
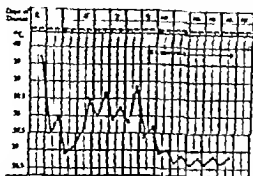
CHART 4 —Temperature chart of a case of infective hepatitis showing continuous fever with a remission on the 4th day

followed by complete recovery are common in Palestine. Such fevers are usually undiagnosed Still the existence of such cases of abortive hepatitis may be presumed on hypothetical grounds

COURSE AND PROGNOSIS.

In the great majority of the cases, the disease runs a benign course ending in recovery within a period of 4 to 8 weeks The duration of the disease is difficult to ascertain. If we take into consideration visible icterus only the average duration in our cases was 28 days the shortest period being 10 days and the longest 90 days. It is realized that this method of estimating the duration of the disease is not satisfactory as the disappearance of the icterus does not indicate the subsidence of the inflammatory process but only a functional recovery. That this recovery may be only partial or temporary is evidenced by the occasional exacerbations noted in some cases. The actual duration is therefore much longer than it is possible to estimate on clinical grounds. The use of the histamine test (KLEIN 1931) and of liver function tests in some cases has proved this contention Unfortunately the technical difficulties involved in the performance of large numbers of tests have prevented us from following this line of research and therefore no statistical information is available.

The prognosis is usually good. There was only one death from the Athlit cases (0 016 per cent). At the Haifa Hospital there were eight deaths during the period 1939-1941 (6 8 per cent). The relatively high percentage at the Haifa Hospital can be explained by the fact that only the more severe cases are admitted

Mode and cause of death All the fatal cases in our series died within the first 2 weeks of the disease In six of the cases both the clinical picture and the postmortem examination were that of acute yellow atrophy of the liver In the remaining two cases there was only a slight icterus the liver was still palpable, and the postmortem examination showed a deeply congested liver haemorrhagic gastritis congested kidneys and ecchymotic spots over the omentum. These findings added to the marked hyperpyrexia and delirium noted for 2 or 3 days prior to the fatal end place these cases in the category of "liver deaths or hepato-renal syndrome" as described by BOYCE (1940).

LABORATORY FINDINGS.

Urine During the initial fever there are no urinary changes with the exception of the occasional findings of traces of albumin. During the hepato-toxic stage there is an increase of urobilin and urobilinogen and as the disease progresses bile pigments appear first, followed by the appearance of bile salts. In the more severe cases, there may be a few granular casts Haemoglobin and red blood cells are not found

Blood The indirect van den Bergh reaction becomes positive in the hepatotoxic stage Later on a delayed direct reaction is present and finally in the more severe cases, both the direct and the indirect reactions are strongly positive.

The serum phosphatase performed in a small number of cases was found to be raised while the cholesterol was usually found to be within normal values The number of cases was so small that no conclusions can be drawn from these findings

Blood counts were made to about 150 cases. The usual finding was that of a leucopenia with a slight monocytosis. Blood counts performed at different stages of the disease are summarized in the following table —

TABLE

	Initial Period			Hepatotoxic Stage		
	Maximum	Minimum	Average	Maximum	Minimum	Average
W B C.	8 600	600	4 342	8 600	2,530	5,435
Segmented	6 %	32%	49°	6 %	36°	53 %
Lymphocytes	45%	22%	33° %	45%	21%	34 %
Monocytes	13 %	2%	8 5 %	13°	%	8 2°

The above table includes only those who eventually recovered In the fatal cases there was a similar blood picture in the initial period but the hepatoxic stage was marked by a leucocytosis from 12 000 to 17,000

The number of leucocytes increases steadily and may reach 35,000 to 40,000 before death. It may therefore be suggested that a leucocytosis in the hepato-toxic stage is a bad prognostic sign.

The blood Wassermann reaction was negative. The sedimentation of the red cells, by the Westergreen method, was found to be normal or slightly raised, giving figures of 10 to 25 mm in the first hour.

DIAGNOSIS.

The diagnosis presents no difficulties in the localities where the disease is prevalent and during epidemics. The sporadic urban cases are the ones that call for special attention. The history of having had "influenza" or "sand-fly fever" shortly before the appearance of jaundice, the history of contact with "jaundice" cases (not often obtained), the history of having visited a locality where infective hepatitis is known to be endemic the blood picture etc., are the only means at our disposal for making a diagnosis. The conditions to be excluded are typhus typhoid malaria cholecystitis cholelithiasis tumours of the liver and pancreas, etc.

HEPATITIS AND PREGNANCY

Infective hepatitis acquires an especially severe character during pregnancy and results in abortion in about two-thirds of the cases. On the other hand there is no evidence that pregnancy increases the predisposition to the disease

RELAPSES AND EXACERBATIONS.

No true relapses were observed. Ten cases were readmitted after periods of 4 to 8 month for what, at first sight appeared to be a relapse. But closer study revealed that though these patients had previously been pronounced cured they had never fully recovered and were now suffering from an exacerbation. These exacerbations tend to occur during the months when infective hepatitis is on the increase.

TREATMENT

There is no known specific treatment for the disease. Prolonged rest in bed is essential. The diet should consist mainly of carbohydrates but sufficient protein supply should be assured. Fats tend to cause gastro-intestinal distress, but this may be prevented by the administration of bile salts. We have given a routine mixture of sodium and magnesium sulphate per os which serves to regularize the bowels.

Intravenous glucose and insulin were used extensively by us but we failed to notice any beneficial effects. In very severe cases vomiting may be persistent and calls for heroic therapy. We have obtained good results with frequent stomach lavages with a weak solution of bicarbonate of soda. In some cases parenteral feeding may be required

During the last few months we used small doses of quinine (0.25 to 0.5 gramme) intramuscularly for 2 or 3 days. The number of cases thus treated was not large, but our impression is that there was a definite improvement in the symptomatology and a shortening of the illness. Two patients who were admitted in a semi-conscious condition with high fever and coffee-ground vomiting definitely improved after quinine and eventually recovered though at first they had been considered hopeless. Further experience with this treatment is required before any definite conclusion can be drawn.

SUMMARY

1. A study of infective hepatitis in Palestine with special reference to its epidemic occurrence at the Athlit Clearance Camp is presented. The study is based on the observation of 633 cases at the clearance camp and of 117 cases at the Government Hospital Haifa.

2. It is concluded that the disease is endemic in Palestine being frequent in the local juvenile population.

3. The clinical picture is described and the clinical course of the disease is divided into four stages namely initial fever intermediate stage hepato-toxic stage, and icterus.

4. The mortality is low. The cause of death is acute yellow atrophy of the liver in the majority of cases and "liver death" or "hepato-renal syndrome" in the others.

5. No specific treatment is known but small doses of quinine seem to be of value.

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also over the Kenya border in Central Kavirondo small foci of the disease persisted for some years. The Budama focus died out some years ago, and no cases had been reported from Samia since 1932.

The evacuated area in Busoga remained empty for many years but for some time previous to the present outbreak natives had been allowed to penetrate in a haphazard manner southwards towards the lake. The result was a series of small isolated outposts of cultivation as each man went back to the lands held by his ancestors. There was no continuity of cultivation, and the people lived for the most part surrounded by dense fly infested bush, and exposed to the damage of game of all kinds. This uncontrolled expansion was, to some extent, limited by declaring a strip of land along the coast to be Crown Forest
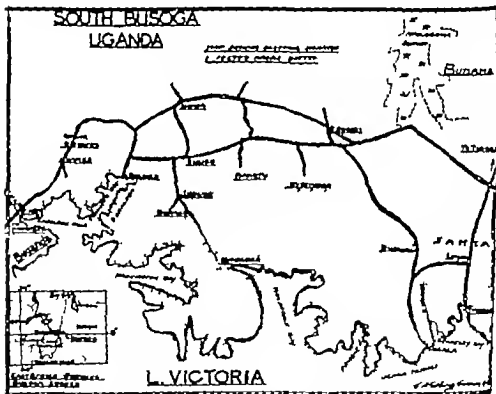
THE COUNTRY AFFECTED

The area involved in the present epidemic of sleeping sickness, that is to say within which all but a very few of the cases occurred, includes the coast line of Lake Victoria from the source of the Nile at Jinja to the Kenya boundary on the Sio river. More recently the island of Buvuma has become infected

and the disease has also crossed over the border into Kenya Inland the area
is roughly bounded by the road running from Jinja to Busia, passing through
Sikiro and Bugiri For the first 8 miles from Jinja this road is never more than
2 miles from the lake and the country consists of densely cultivated banana and
cotton plots with a few areas of regenerating bush This gives way to the
sugar estates at Kakira which extend for about 8 miles inland with a narrow
cleared frontage on the lake shore After this belt of cane the country south
of the road consists of dense uninhabited bush with areas of cultivation extending
to a greater or lesser extent from the road. Secondary roads run south to the
lake at Buluba, Kiterera (now closed) Lugalla and Mjanji Settled areas
extend along these roads and also along those running to Makutu and Kwemeire
a distance of about 6 miles in each case In short, the country between Buluba
and Mjanji consists of a large uninhabited tract, about 400 square miles in
extent, with a fringe of cultivation along the northern edge and a rather thicker
belt enclosing it to the east.

In the west, where the rainfall is higher the vegetation consists of thick
rain forest of the semi-evergreen type. This mainly occurs along the ridges
between which are valleys of open acacia bush and nearer the lake occasional
papyrus swamps intruding for a short distance inland. In the east, where
conditions are drier large tracts of *Combretum* savannah are found interspersed
with dense clumps of deciduous thicket running along the slight ridges which
lie east and west. There are no hills of any height, the highest being Bukaleba
(4 473 feet)

There are no permanent rivers and a feature of the country is the fact
that all the water up to a mile or two from the lake drains northwards along
swamps to the Mpologoma Swamp and Lake Kioga. Water is mainly derived
from ironstone pans and waterholes in swamps during the rains The rainfall
for most of the area is over 50 inches per year

Game is very plentiful elephant and buffalo being found throughout in
addition water buck (*Kobus defassa* ssp) bushbuck (*Tragelaphus scriptus* ssp)
duiker (*Cephalophus caerulus* ssp) sitatunga (*Limnotragus spekii*) and kongoni
(*Alcelaphus buselaphus* ssp) are all found Bush pig (*Potamochoerus porcus* ssp)
are very common and do an immense amount of damage to crops Near the
lake hippopotamus crocodile, and monitor lizard (*Varanus niloticus*) are all
common. No cattle are kept in the area and goats are not numerous A few
dogs do manage to exist and are used for hunting pig in conjunction with
nets and spears It has been the practice for some years now to burn the
grass only in December i.e early in the dry season, in order to limit the
fierceness of the fire and protect valuable timber

The population consists for the main part of Basoga, a Bantu people
Their food is made up of plantains as the staple diet, with the addition of
ground nuts, beans rice, and fish or meat. Cotton is grown as the main cash
crop They are not enthusiastic hunters and on the whole prefer fish to meat.

In the east are the Samia tribe, often referred to inaccurately as Kavirondo. They are a Nilotic people, originally large cattle owners, but have lost their former herds through the ravages of tsetse. They live partly in Kenya and partly in the Mbale district of Uganda between Busia and Mjanji. Of late on Gombololo, Buswale, has been incorporated in Busoga. Their staple food is *eleusine* but they are primarily fishermen, and before the present outbreak carried on an extensive fishing industry from Mjanji and Lugalla. They are also hunters and consume a large quantity of meat.

HISTORY OF THE PRESENT OUTBREAK.

The current epidemic dates from November 1940 and up to the middle of 1943 there were 2,432 cases with 274 deaths.

The first case was diagnosed at Kampala, in a school-boy from Busoga who was visiting there. He was taken ill and trypanosomes were found in his blood. His previous history was confused but it appears that he had visited the neighbourhood of the lake, somewhere between Iganga and Jinja, on his way to Kampala. It is almost certain that he was infected in that area, since his home was in a part of Busoga free from fly. Two further cases were discovered in Jinja Hospital in the same month both were Banyaruanda employees of the Kakira Sugar Estates, 8 miles from Jinja. In the following month December two more cases were found at Jinja one a Munvaruanda labourer from Kakira and the other a local Musoga from the lake shore on the outskirts of Jinja township. So, of the first five cases, three were alien natives, from Ruanda Urundi Territory employed at Kakira.

Measures were then taken to safeguard the population near Jinja and to try to arrest the course of the disease. It was thought at that time that the most likely source of the sickness was to be found among the Kavirondo fishermen who were then living along the lake shore between Jinja and Kakira. These were all potential carriers, as they all came from Samia or Kenya, where there was still an active focus of the type of sleeping sickness caused by *Trypanosoma gambiense*. All of these aliens were quickly repatriated, and the rest of the shore between Kakira and Jinja was also evacuated up to the main road, a distance of 1 to 2 miles in depth. Large clearings were made through this evacuated area, by which the inland population could draw water from the lake, while boreholes to provide an alternative supply were being sunk along the road.

By the end of March, 1941 these measures had succeeded in stamping out the disease in this area, although cases were still occurring among the Kakira labourers. In that month, however a report was received of an outbreak of illness among the inhabitants of the Leper Colony at Buluba, situated at the head of Thruston Bay about 12 miles east of Kakira. Investigation showed that they were suffering from sleeping sickness, and in the next 8 months twenty cases in all were found there.

In April of the same year a fresh focus was found still further east, among the forestry workers employed in the Kiterera area so that by June the total number of cases had risen to eighty

In view of the danger of further spread a survey was begun in July, 1941, of the whole south Busoga coastal area, and of the fly belts along the Nile and Mpologama swamp Evacuation of the people, big clearings and prophylactic injections of antrypol had by now been enforced as far as Buluba and no more cases were now coming from that area, though the Kakira focus was still not finished. The survey, however disclosed a large number of cases in the Kiterera and Ikulwe areas, showing that the disease had got a definite hold there and had involved the inland population far from the lake shore or any permanent water This was puzzling in view of the belief then held that *Glossina palpalis* was responsible for the spread but it seemed to be satisfactorily explained by the discovery of this fly in large numbers in the dense inland thickets The rest of the population living within 6 miles of the lake was also examined as far east as the Kenya border, without discovering any further cases

The situation thus appeared stable, and as it seemed that the infection was self limited by the inland extent of the fly belt evacuation of the Ikulwe and Kiterera areas to beyond the estimated range of *G palpalis* (in some cases as far as 7 or 8 miles from the lake) was begun In November of that year however the situation was changed as the sickness flared up again 20 miles to the east at Kyemeire and at least 12 miles from the lake. From there it spread rapidly until all the rest of the area south of the main road was involved, including places which had been examined only 2 or 3 months previously and found apparently free. Within the next 2 months the epidemic had spread through Samia and over the Sio river into Kenya.

In order to deal with the large number of infected persons now coming in for treatment, a temporary camp was built at Bugiri for their accommodation Cases were arriving at the rate of over a hundred a week at the peak of the epidemic, which was reached in March, 1942 The cases then in the camp were more than a thousand.

After that the cases dropped steeply in number until at the present time the average is between ten and twenty per month for the whole of the area. It is hard to say what caused the unexpectedly sharp decline which took place before all the present measures could be put into effect, but perhaps the regression of the fly with the cessation of the abnormally heavy rains, together with the rapid removal of infected persons into camp and away from contact with the tsetse may both have contributed.

CLINICAL FEATURES.

The most striking feature of the epidemic, especially when at its peak, was the virulence and rapid course of the sickness as contrasted with the

T gambiense infection. Within 4 to 6 weeks of the onset persons attacked were in an advanced stage of the disease and many were moribund on admission to the camp Extreme emaciation and weakness were often seen before death very rarely were any signs of nervous involvement observed

In the earlier stages oedema was a common symptom varying from an extreme almost renal, type in young children to slight oedema of the ankles in other cases. The face and upper lids were most commonly affected in young adults and children, and the feet and ankles in older persons. Thus, of course, was quite possibly due to the anaemia present at the time. Terminal ascites was seen in a few cases. A peculiar feature of the majority of deaths was the very high percentage of persons complaining of diarrhoea and abdominal pain a day or two before death. Once these symptoms set in there was but little hope of recovery Investigations showed that this diarrhoea apparently bore no relation to the clinical state, or to the type or amount of the drugs used It is possible that it was due to some kind of bacillary dysentery in the camp though the same thing was observed among individual cases treated in hospital elsewhere *Examination of the stools showed the usual helminthic infestations* A possible light is thrown on this by the publication of detailed accounts of postmortem examinations of cases dying from *T rhodesiense* infections (HAWKING and GREENFIELD 1941) In this report the occurrence of lesions in the bowel and peritoneum is stressed, such as might have given rise to the terminal dysenteric symptoms mentioned above. Unfortunately no postmortem examinations could be carried out in Busoga and Samia. The people affected by this epidemic are all profoundly superstitious and distrustful of hospitals and any suspicion of interference with the dead would have caused wholesale desertion from the camps and concealment of sickness

Symptoms of involvement of the central nervous system were very rare but examination of the cerebrospinal fluid showed that in about 25 per cent. of the cases the cell count was over 100 showing the early involvement of the system by the disease.

No typical rash was observed. The cervical and axillary glands were only very occasionally of the large soft type suitable for gland puncture, such as are found in the *T gambiense* type of sleeping sickness.

The early diagnosis of these cases by clinical methods is not easy In most cases the cervical glands are affected, but this is commonly due to other causes, and enlargement of the axillary and epitrochlear glands proved a much more reliable diagnostic sign. The serum-formalin test was tried but proved unreliable, and it was found that microscopical examination of the blood of all exposed persons with any symptoms at all gave the most satisfactory results and was the most practical method of finding early cases.

At present a system of examination by travelling teams equipped with microscopes is in operation throughout the area and ensures that everyone is examined at least once in every 3 months. This is supplemented by aid posts

and dispensaries where the people are encouraged to come for treatment for any kind of illness and where blood examinations can be carried out. Under this system most of the cases are found in the early stages and their number has shown a progressive decline

TYPE OF TRYPANOSOME INVOLVED

In December 1941 it was first found possible to investigate the type of trypanosome involved, up to that time it was thought that one was dealing with a strain of *T gambiense*

Twenty rats (both white rats and the local form of *Rattus rattus*) and two guineapigs were injected with the blood of positive cases chosen from all parts of the infected area and every part of the country between Jinja and Kenya The method of injection employed consisted of withdrawing 1 c c of blood from the patient's vein and inoculating the rat intraperitoneally with this blood. The rats were all examined before injection to see that the blood was free from trypanosomes In one case *T lewisi* was found in a wild rat the others were all negative

Following the injection, trypanosomes were found in the rats blood on the 5th or 6th day and posterior nucleate forms 2 or 3 days later on the average Two rats failed to take the infection and another died before becoming positive The posterior nucleate forms were numerous from the first days of their appearance rough counts of the proportion of these to other forms were made at various times and the percentage was found to vary between 5 and 12 In many of these the nucleus was posterior to the kinetoplast, and the trypanosomes showed great variations in size and shape. The infection proved rapidly fatal to the animals the rats dying between 4 and 5 weeks after the injection and the guineapigs 3 or 4 weeks later

Having regard, then, to the early and profuse appearance of posterior nucleate forms following the first inoculation, and the rapid and fatal course of the disease in the animals used it is reasonable to believe that the strain is identical with *T rhodesiense* This is apparently the first time that this strain has been isolated in Uganda there is a record of a previous case in the Western Province being diagnosed as of the *T rhodesiense* type but there is no evidence that animal inoculation was performed.

It is unfortunate that the strain could not have been studied at the beginning of the outbreak, but one was misled by the fact that *G palpalis* seemed the obvious carrier and by the nature of some of the early cases, at least two of which were more typical of infections with *T gambiense* It is also possible that at the beginning a mixed infection of the two strains was present.

The fact that the population lived for many years in close contact with fly and game presumably infected with *T brucei* without contracting sleeping sickness, and then suddenly succumbed to a widespread epidemic, seems to

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Lugalla area dissected out the salivary glands of both *G palpalis* and *G pallidipes.* He found trypanosomes in the glands of both species, his figures being as follows *G palpalis* 0 8 per cent. glands infected of 509 flies examined. *G pallidipes* 0·33 per cent. glands infected of 603 flies examined.

The dissected glands containing trypanosomes were suspended in saline and inoculated intraperitoneally into white rats. Two of the rats became infected, one with trypanosomes derived from *G palpalis* and the other with a strain from *G pallidipes* The trypanosomes seen in blood smears from both these rats were polymorphic and showed many posterior nucleate forms, as is typical of the *T brucei rhodesense* group In addition the rats were quickly killed by the disease. Unfortunately it was not found possible at the time to inoculate either of these strains into human volunteers.

In April, 1943, Dr C H N JACKSON of the Department of Tsetse Research, Tanganyika Territory was lent to the Uganda Government to conduct further investigations into the carrier problem He collected numbers of *G pallidipes* from the Lugalla area and fed them on white rats. This area was the site of numerous cases in 1941–42, but had been cleared of all inhabitants at least 1 year before the flies were caught. The nearest human source of trypanosomes was about 3 miles distant from where the flies were found, and the only persons entering the area were very occasional trespassers in search of fish and game.

The flies thus obtained were allowed to feed in batches of 100 on each of fifty rats. Out of all the flies used it was estimated that about 3,500 fed, so that each of the rats received an average of approximately seventy bites On completion of the feeding the flies were all killed and examined one *G palpalis* was found and the rat in question was discarded. The rats were a clean strain obtained from the Veterinary Laboratories, Entebbe and were examined before the experiment was begun.

Of these rats, five showed infection with a polymorphic trypanosome of the *T brucei* group after 7 days and twenty two others became infected with *T congolense* 12 days after the flies had fed. The five rats infected with polymorphic trypanosomes were killed. From the heart of each rat 0·25 c.c. of blood was taken, mixed with 0·25 c.c of sterile normal saline, and injected into the arm of each of five human volunteers, 0·25 c.c. of the mixture being given subcutaneously and the remainder intramuscularly Four of these volunteers showed no reaction at all and their blood was negative up to the 10th day after the inoculation, when they received a precautionary course of antrypol. The other man developed a painful swelling of his arm around the site of the injection on the 4th day His temperature also began to rise, and on the 5th and 6th days reached 102° F In the afternoon of the 6th day scanty trypanosomes were found in the peripheral blood. He was at once given an injection of 1 gramme of antrypol. Trypanosomes were still present in the blood the following morning but disappeared later in the day His temperature began to fall at once and his arm cleared up rapidly The trypanosomes in his blood

smears were all morphologically similar to those found in cases of sleeping sickness. This volunteer was an employee of the Medical Department and lived in Jinja out of contact with the fly.   In any case the coincidence of symptoms is such that there can be no doubt that he received his infection as a result of the injection.

The fact that the other strains of trypanosomes failed to infect the volunteers is capable of two explanations either they were an innocuous strain of *T brucei* or else they were *T rhodesiense* but in a non infective state It is known that *T brucei* exists in the area, since some years ago this trypanosome was isolated from the blood of a sick dog at Kiterera and more recently two dogs in Jinja have been found infected with a similar trypanosome

Regarding *G palpalis* as a carrier of *T rhodesiense* some work has already been done, and more is in progress. About 400 flies from the Kiterera area were caught and fed on nine rats, in which two strains of the polymorphic trypanosomes and two of *T congolense* developed. One of the polymorphic strains was inoculated into a human volunteer as above, but failed to produce an infection. The other strain was unfortunately, lost owing to the sudden death of the rat.

Each of a further thirty two rats was also fed upon by an average of seventy-five *G palpalis* from the infected area of Buvuma Island From these, two further strains of polymorphic trypanosomes were isolated one was sent to the Sleeping Sickness Research Laboratory at Tinde Tanganyika Territory where it was inoculated into a succession of volunteers without result. The other strain was lost owing to a dearth of local volunteers These experiments have recently been repeated, thirty-eight rats being fed upon by an average of fifty *G palpalis* from the Buluba and Kiterera areas. No strain of trypanosome was obtained from these flies

ORIGIN OF THE EPIDEMIC

Setting aside the possibility of mutation of indigenous strains of *gambiense* or *brucei* some source outside Uganda must be sought. The nearest areas where *T rhodesiense* is endemic are to be found in Tanganyika, though the occurrence of cases in the south of the Sudan has been reported. The most likely route by which the infection might have reached Uganda from Tanganyika is along the west side of Lake Victoria. It has been pointed out that the early cases were from the neighbourhood of the Kakira sugar estates near Jinja. These employ about 9 000 labourers mostly Warundi, Wanyaruanda or Waha, from the Ruanda Urundi territory of the Belgian Congo and all from areas in reasonable proximity to infected parts of Tanganyika. It is significant that of the first three cases of sleeping sickness two were natives of this kind, and that early in the epidemic two cases admitted to Jinja Hospital from Buganda were both members of the above tribes who had fallen sick while still on their

way to Busoga to work. On this evidence then it seems most likely that the infection was introduced from Tanganyika to Busoga by this route.

SUMMARY

The occurrence of an epidemic of sleeping sickness involving 2,500 persons in the Busoga and Samia districts of Uganda is described.

Proof is given that the trypanosome responsible is *T rhodesiense*

Epidemiological and experimental results point to *G pallidipes* as the principal vector The part played by *G palpalis* is still uncertain.

Experiments are described which prove that *G pallidipes* can carry naturally a trypanosome of the *brucei rhodesiense* group which is capable of causing sleeping sickness in man.

It is probable that the infection was introduced by alien labourers from the west of Lake Victoria.

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STUDIES IN LEISHMANIASIS
IN THE ANGLO EGYPTIAN SUDAN

VI.—THE EVOLUTION OF LEISHMANIA INFECTIONS IN MAN

BY

R. KIRK M.O

Sudan Medical Service

In recent years attention has been attracted to cutaneous and mucocutaneous leishmaniasis occurring in the kala azar areas of the Sudan and the various types of infection observed there have been described in previous papers A proportion of the cases described in those papers had also visceral kala azar at the time the dermal condition was recognized   In such cases difficulty arises in determining whether the skin condition is caused by the parasites of kala azar or by superimposed infection with oriental sore since it has been found that purely cutaneous infections may occur in the endemic areas of the Sudan. In a number of instances we resorted to animal inoculation in the hope that it might help to elucidate this question. Parasites from dermal and visceral conditions were inoculated by various routes into white mice and monkeys (*Cercopithecus aethiops* and *Erythrocebus patas*)   The results were so variable, and included so many failures to produce any type of infection that no useful information regarding differentiation of strains has been obtained by this method. Perusal of the literature shows that other workers have had similar experiences   Differentiation of strains by animal inoculation is difficult unless large numbers of suitable animals are available while strains of *Leishmania tropica* which are apparently similar in the human subject may produce very divergent results when inoculated into animals.

Although the results of animal inoculation proved disappointing a ain amount of information has been obtained from the study of naturally or attracted infections in human beings. The position in the Sudan is of whcular interest in this connection since kala azar nasopharyngeal leish occmisiasis and dermal conditions resembling oriental sore may all be found like the same endemic area. The picture is further complicated by the occurrence of skin infections resembling the post kala azar dermal leishmaniasis of India cases a proportion of successfully treated cases of kala azar   In a previous com Thenication (KIRK, 1942) summarizing the observations of some 8 years frondence was produced that although there are infections in the Sudan com in rable to oriental sore in that they produce only cutaneous lesions, without of bsequent visceral involvement, the parasites of Sudan kala azar may at times cause also cutaneous and nasopharyngeal leishmaniasis   The scope of that communication was restricted closely to infections contracted in the

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way to Busoga to work. On this evidence then it seems most likely that the infection was introduced from Tanganyika to Busoga by this route.

SUMMARY

The occurrence of an epidemic of sleeping sickness involving 2,500 persons in the Busoga and Samia districts of Uganda is described.

Proof is given that the trypanosome responsible is *T. rhodesiense*.

Epidemiological and experimental results point to *G. palhdipes* as the principal vector. The part played by *G. palpalis* is still uncertain.

Experiments are described which prove that *G. palhdipes* can carry naturally a trypanosome of the *brucei rhodesiense* group which is capable of causing sleeping sickness in man.

It is probable that the infection was introduced by alien labourers from the west of Lake Victoria.

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STUDIES IN LEISHMANIASIS
IN THE ANGLO EGYPTIAN SUDAN

VI —The Evolution of Leishmania Infections in Man

BY

R. KIRK, M.D

Sudan Medical Service

In recent years attention has been attracted to cutaneous and mucocutaneous leishmaniasis occurring in the kala azar areas of the Sudan and the various types of infection observed there have been described in previous papers A proportion of the cases described in those papers had also visceral kala azar at the time the dermal condition was recognized In such cases difficulty arises in determining whether the skin condition is caused by the parasites of kala azar or by superimposed infection with oriental sore since it has been found that purely cutaneous infections may occur in the endemic areas of the Sudan. In a number of instances we resorted to animal inoculation in the hope that it might help to elucidate this question. Parasites from dermal and visceral conditions were inoculated by various routes into white mice and monkeys (*Cercopithecus aethiops* and *Erythrocebus patas*) The results were so variable and included so many failures to produce any type of infection that no useful information regarding differentiation of strains has been obtained by this method. Perusal of the literature shows that other workers have had similar experiences. Differentiation of strains by animal inoculation is difficult unless large numbers of suitable animals are available while strains of *Leishmania tropica* which are apparently similar in the human subject may produce very divergent results when inoculated into animals

Although the results of animal inoculation proved disappointing a certain amount of information has been obtained from the study of naturally or attracted infections in human beings The position in the Sudan is of peculiar interest in this connection, since kala azar nasopharyngeal leishmaniasis and dermal conditions resembling oriental sore may all be found in the same endemic area. The picture is further complicated by the occurrence of a skin infections resembling the post kala azar dermal leishmaniasis of India in a proportion of successfully treated cases of kala azar In a previous communication (KIRK, 1942) summarizing the observations of some 8 years, evidence was produced that although there are infections in the Sudan comparable to oriental sore in that they produce only cutaneous lesions, without subsequent visceral involvement, the parasites of Sudan kala azar may at times cause also cutaneous and nasopharyngeal leishmaniasis The scope of that communication was restricted closely to infections contracted in the

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Sudan, and studied personally by the writer   Subsequent observations have strengthened the opinion, then suggested, that in the Sudan true nasopharyngeal leishmaniasis is often a post kala-azar condition.   The writer believes that he has seen leishmania infections in the human subject going through a fairly specific course of evolution, the various stages of which are comparable with well recognised conditions of leishmaniasis which occur in other parts of the world.

The present communication is essentially a discussion of this hypothesis. It is not restricted in its scope to facts observed personally by the writer but includes comments on papers from the other endemic centres of leishmaniasis which are selected and interpreted in the light of the writer's personal opinions, based primarily on conclusions drawn from his own observations in the Sudan.

MANIFESTATIONS OF LEISHMANIA INFECTION IN MAN

The classical forms of leishmaniasis, oriental sore, kala-azar espundia, and dermal leishmanoid are distinct clinically   Our observations in an area where all forms are encountered indicates however that intermediate and transitional forms are occasionally seen.   It is evident from the literature that workers in the other endemic centres of leishmaniasis have observed transitional forms similar to those which we have seen in the Sudan.   It has been shown in India that dermal leishmanoid is essentially a post kala-azar condition.   Russian workers have now demonstrated that an inconspicuous primary cutaneous lesion occurs in kala-azar as was postulated some years ago by NAPIER and KRISHNAN (1931) in India, and there is evidence that nasopharyngeal lesions of the espundia type are in some instances secondary or tertiary manifestations of leishmanial infection.

It is suggested that leishmania infections tend to undergo a fairly specific course of evolution in the human subject, characterized by three stages —

   I   Primary stage—cutaneous sores at the site of inoculation, having a tendency to spontaneous cure.

   II   Secondary stage—generalized infection kala azar

   III Tertiary stage—cutaneous and mucocutaneous infection.

All stages are not seen in a single infection, partly because different strains of leishmania vary greatly in virulence   The infection may be terminated at any stage by the defence mechanism of the host gaining the upper hand, with the development of complete immunity and suppression of the parasites. Or it may end with the death of the patient in the stage of generalized infection. Further in any given infection one particular stage or lesion may be prominent, while another may be inconspicuous, or difficult to demonstrate.   Some of the lesions, or types of infection, on which the views expressed in this paper are based have not, in fact, been recognized until quite recently

I. PRIMARY STAGE—CUTANEOUS LESION

ORIENTAL SORE.

In oriental sore the principal feature of the infection is a cutaneous lesion,

containing leishmania, at the site of inoculation The typical lesion can be reproduced by direct inoculation of material from one patient to another In the naturally acquired infection the lesion may be single or multiple, and many different clinical types have been described. As a rule the visible results of the infection are entirely cutaneous, and its normal course is to undergo spontaneous cure after a period of several months with the development of immunity to further infection with the same strain of parasite

KALA-AZAR.

In kala azar the development of a primary lesion at the site of inoculation has not generally been observed. There is nevertheless, evidence that it occurs ARCHIBALD (1922) noted that when monkeys are inoculated subcutaneously with material from cases of Sudan kala azar the development of the visceral infection is sometimes preceded by the appearance of a skin lesion of the oriental sore type at the site of inoculation. The writer has similarly observed a cutaneous lesion in a monkey inoculated from a splenic puncture but in this instance a visceral infection failed to develop later or was not recognized.[*] In an attempt to explain the epidemiology of kala azar in India, NAPIER and KRISHNAN (1931) postulated that the first stage of the disease is an inconspicuous focus of infection at the site of inoculation the further course of the infection according to those authors, is determined by the resistance of the host and a number of coincidental factors which induce migration of infected histiocytes from the focus of infection in the skin to the internal organs thus leading to visceral infection

Some years ago the present writer (KIRK 1938) depicted an inconspicuous superficial scaling lupus-like lesion, which began as a small papule on the left malar eminence, as a primary sore in a case of Sudan kala azar A few other similar instances have been noticed in the Sudan (KIRK 1942) in one of which the lesion occurred, not on the face, but on the anterior surface of the leg At the time they came under observation those patients all had kala azar and there was only presumptive evidence (from the history) that the appearance of the cutaneous lesions had preceded the onset of the visceral disease. To show that the cutaneous lesions were in fact primary manifestations of kala azar it would have been necessary to prove conclusively that their appearance antedated the onset of visceral symptoms, and also to exclude coincident infection with oriental sore This was beyond the facilities at our disposal in the Sudan, but it has apparently been done recently by MIRZOIAN (1941) in Central Asia From observations continued over 2 years on eighty children in Samarkand this author concludes that several months before kala azar can be diagnosed clinically the primary lesion in children in this area

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[*] The course of the visceral infection in *Cercopithecus aethiops* is greatly influenced by diet and general living conditions. A fatal termination is the rule in the ordinary conditions of captivity in cages in the laboratory But if the animals are taken out of their cages given a large amount of freedom and a varied, nutritious diet with abundance of fresh fruit, spontaneous recovery often occurs.

is manifested in the form of one or more papules, about the size of a pin head, appearing on the face. They increase to the size of a lentil when they are pink or dark red in colour and disappear several months later leaving pigmented spots. Early papules are not associated with palpable cervical glands, but these later increase in size, and subsequently the spleen and other lymph glands become enlarged. By the time kala-azar is clinically recognisable the parasites may be present simultaneously in the skin lesions and in sternal puncture material (50 per cent. of cases) or they may have disappeared from the former. The lesions are quite similar to that described by the writer from the Sudan, but they may be very inconspicuous. They are best seen in children under 2 years of age with tender skins and clear complexions. In older children they may be more difficult to detect owing to the roughness and pigmentation of the skin.

LEISHMANIASIS AMERICANA.

Cutaneous infection features prominently in the condition known as *American leishmaniasis*, in which the parasites have a special tendency to attack the mucous membranes of the nose, mouth and pharynx. ESCOMEL (1916) differentiated uta as a form of this disease in which the infection is essentially one of the skin of the face and in which the ulceration extends peripherally from the primary sore to the oro-nasal mucous membranes without any breach of continuity. In true espundia, on the other hand, the first evidence of the disease is one or more cutaneous lesions of the oriental sore type, occurring on the exposed parts of the body. These eventually heal, and are followed later by ulcerative lesions of a most intractable character in the nasopharynx, so that there is an interruption of continuity between the primary cutaneous sore and the later manifestations. There is evidence that in many instances of this infection the later manifestations never develop, and the condition ends with the cure of the primary cutaneous lesion.

II SECONDARY STAGE—GENERALIZED INFECTION.

KALA-AZAR.

In kala-azar the infection becomes generalized. The method or routes by which it does so are somewhat uncertain. NAPIER and KRISHNAN (1931) have suggested that the occurrence of some superimposed condition may be responsible, such as malaria or typhoid, which causes a reaction in the reticulo-endothelial tissues and mobilization of large mononuclears in the blood. Infected cells are thus carried from the primary focus in the skin to the visceral reticulo-endothelial tissues, and a general visceral infection becomes established. KIRK and SATI (1940) have suggested that lymphatic spread may play a large part in the dissemination of infection throughout the body and the observations of MIRZOIAN (1941) lend some support to this view. Probably several methods of spread are involved, but the principal factor

determining whether or not visceral infection will occur is the type or strain of parasite with which the patient is infected

The essential feature of the generalized infection is an invasion of the reticulo-endothelial tissues in all parts of the body by leishmania, with great proliferation of reticulo-endothelial cells. Parasites are most readily found in the spleen, lymph glands, and bone marrow but in addition most of the other organs in the body may become involved. We have found parasites in the liver, kidneys, suprarenals, pancreas, lungs, intestine and testes. Other observers have recorded their occurrence in thyroid, thymus, heart, stomach, prostate, cerebrospinal fluid, arachnoid, and serous membranes. They may also be found in the blood.

The course of the generalized infection is in 90 per cent of cases that of textbook kala-azar and need not be described here. In the Sudan two extreme variations from the typical course are sometimes seen. The first is a very acute form of the disease, with sudden onset, high fever, severe constitutional symptoms, little or no splenic enlargement and, in the absence of treatment, rapid progress to a fatal termination. Such cases usually react well and rapidly to treatment if the diagnosis is made early enough. The other type is an exceedingly chronic form of the disease with large stony hard spleen and little or no constitutional disturbance other than an occasional attack of fever. Parasites are usually difficult or impossible to find even in repeated spleen punctures, but sometimes they may be found readily and in large numbers. In such cases the results of treatment are often disappointing and the ultimate prognosis uncertain.

ORIENTAL SORE.

In oriental sore occasional cases have been noted in which the occurrence of an obscure febrile condition suggested the possibility of a transient general infection (MANSON 1917) but this has not been proven. Sometimes but not commonly there may be adenitis associated with the presence of leishmania in the related lymph glands. Leishmania have been found in the peripheral blood in cases of oriental sore by NEUMAN (1909) and PATTON (1911) but other workers have consistently failed to confirm this. As a rule the visible results of the infection are entirely cutaneous. The fairly lasting immunity conferred by an attack of the disease is interesting however since it apparently involves the whole skin, thus suggesting that something more than a mere local immunity reaction has been evoked.

LEISHMANIASIS AMERICANA

The writer has seen no published work which indicates clearly a stage of generalized infection in leishmaniasis americana. There is much confusion in the literature on this subject. Apparently there are many different strains of leishmaniasis in South America. In the typical espundia of the textbooks however it is stated (MANSON BAHR, 1935) that although the abdominal viscera

are not affected the lymphatic glands may become involved, while the literature referring to this condition indicates that the nasopharyngeal ulceration is in many instances the late result of an infection which started as a spontaneously healing cutaneous sore on some other part of the body It is difficult to see how the parasites can initiate mischief in the nasopharynx, sometimes many years after the primary lesions have healed completely without some form of systemic migration having occurred Perhaps further studies in the recently discovered visceral leishmaniasis of South America will help to elucidate this question.

III TERTIARY STAGE—CUTANEOUS AND MUCOCUTANEOUS INFECTIONS.

POST KALA AZAR DERMAL LEISHMANIASIS

This was first recognized in India by BRAHMACHARI (1922) who showed that the condition was a sequel of visceral kala azar which had been successfully treated with antimony It has since been extensively studied in India by ACTON and NAPIER (1927) NAPIER and DAS GUPTA (1930), and other workers, while the present writer has reported closely similar dermal conditions which occur in Sudan kala azar patients after successful treatment. It is a very curious fact that complete and permanent cure of the visceral infection is apparently compatible with an extensive invasion of the skin by the parasites, where they may produce cutaneous lesions after a latent period of 1 to 2 years in Indian kala-azar or in the Sudan variety just after the completion of treatment. This residual or post kala azar infection has to be differentiated clearly from incomplete cure. In the latter relapse of the visceral disease is likely to occur whereas all the evidence indicates that visceral relapse is exceedingly rare once the dermal condition has become established.   There is some evidence also that post kala azar dermal leishmaniasis is associated with immunity to reinfection. The condition occurs typically in treated cases, but has been found in rare cases of apparently spontaneous recovery from the visceral disease, thus suggesting that it is associated with an immunity response of some kind.

Post kala-azar dermal leishmaniasis is protean in its manifestations, and many clinical types have been described. The lesions occur commonly on the face, but they may be found on any part of the body They may be all over the body and are quite commonly widespread. The mechanism by which the change from visceral to dermal infection occurs is still imperfectly understood. NAPIER (1935) marshals some very suggestive evidence that it is the result of a generalised dissemination of parasites in the blood stream, which occurs during the visceral phase. This view is entirely consistent with our observations in the Sudan, where the development of the dermal infection can sometimes be recognized before the signs of the visceral disease have completely subsided and is regarded by the writer as sound evidence that the case is progressing satisfactorily In Indian kala azar on the other hand, there is a latent period of about a year between the subsidence of the visceral disease

and the appearance of the skin lesions during which period it has to be presumed that the parasites are lying dormant in their dermal situation without causing lesions.

From the sanitarian's point of view a patient with post kala azar dermal leishmaniasis is practically a carrier. The lesions may be very inconspicuous yet persist for a very long time—over 20 years in a case described by the writer (KIRK, 1942). General health is unaffected and there is little tendency to visceral relapse. But owing to the situation of the infection in the skin, sand flies may readily become infected by feeding on such an individual. In a place where suitable vectors are abundant he may infect large numbers of them and thus be a source of danger to other people. NAPIER (1935) has suggested that obvious clinical manifestations occur only in a proportion of those who develop skin infections after recovery from kala azar with or without treatment. Others pass into a true carrier stage in which the skin infection remains entirely subclinical yet infective for sandflies.

The phenomenon of tertiary skin infection in successfully treated cases is also of great theoretical interest, since it provides some indication of the manner in which drugs act to produce cure in kala azar. EHRLICH's original conception of the action of chemotherapeutic agents was that of magic bullets —selective poisons killing only the parasites by virtue of a highly specific affinity for certain chemical groups or constituents of the cells attacked with which they formed firm combinations. The trend of modern work on the other hand, is to regard the action of the most successful chemotherapeutic agents as usually a mild and persistent one depending in some instances, as do the pentavalent arsenicals, on affinity for the host's tissues as well as the parasites; in others like the sulphonamides on interference with specific biochemical factors necessary for the growth and reproduction of the parasites. In kala azar something more than a lethal action of the drug on the parasites is required to explain the observed phenomena. Complete clinical cure of the visceral disease is apparently consistent with extensive invasion of the skin by the parasites, where they may persist for many years afterwards. The effect of treatment appears to be one of restraining or inhibiting the parasites rather than eliminating them completely or turning to their defeat the tide of battle with the host's resistance during the stage of visceral infection. In addition some profound and lasting change in the host parasite relationship occurs during treatment, since once the dermal infection has become properly established there appears to be little or no tendency to visceral relapse even after long intervals with no further treatment. Although the parasites may not be eliminated under the influence of chemotherapy the course of evolution of the infection becomes in fact, similar to that which has been observed in spontaneous recovery. From our observations in the Sudan it can be stated that this is so in cases treated with the diamidines as well as in cases treated with antimony—a point of some interest, since the diamidines and antimonials are entirely different from each other in chemical constitution.

NASOPHARYNGEAL INFECTIONS

Infections of the mouth and nasal cavities may occur in any stage of leishmania infection. Many such conditions are evidently due to oriental sore, in which the site of inoculation just happens to be near the margins of the nose or mouth, and the ulceration extends directly from the primary lesion to those parts. It is curious, however that involvement of the mucous membranes seems to be associated with a much more intractable form of ulceration than is usual with oriental sore on other parts of the body.

In kala azar it has been shown by FORKNER and ZIA (1935) that leishmania can often be found in nasal and tonsillar smears and their observations have been confirmed by others. Past experience in the laboratories in Khartoum (HORGAN 1944) has also shown that heavy infections of leishmania are commonly found in the nasal smears from experimental *Cercopithecus* monkeys with visceral kala-azar. The writer (KIRK, 1942) has shown that definite ulcerative lesions in the nose and mouth may be associated with concurrent visceral involvement in Sudanese kala azar patients.

Finally there is considerable evidence that true nasopharyngeal leishmaniasis is frequently a tertiary or post kala azar condition. NAPIER and GUPTA (1930 1934) DISTIDAR (1939) and other Indian observers have depicted ulcerative conditions of the lips, palate and tongue as clinical varieties of post kala-azar dermal leishmaniasis. A case has been recorded from the Sudan (KIRK and MACDONALD 1940) in which an intranasal ulcer containing leishmania appeared simultaneously with a nodular skin eruption after treatment of kala-azar with antimony. Two other cases of Sudan kala-azar have been seen subsequently by the writer (unpublished) in which the development of a nodular eruption at the end of treatment coincided with the appearance of purplish patches on the palate and fauces, containing leishmania. As regards leishmaniasis americana there is evidence that in some cases at least the extensive nasopharyngeal ulceration found in that condition is a secondary or tertiary manifestation of an infection which began sometimes many years earlier as a spontaneously healing cutaneous sore on some other part of the body (LINDSAY 1917).

DISCUSSION

It will be noted that apart from the inclusion of some forms of nasopharyngeal leishmaniasis with the tertiary skin manifestations this conception of the way in which leishmania infections evolve in the human subject is essentially the same as that postulated by NAPIER and KRISHNAN (1931) to explain the epidemiology of kala-azar in India. Moreover in the Sudan the writer has seen all the clinical manifestations mentioned, and believes that he has personally observed the evolution of the infection occurring in different individuals through the various stages described.

It is not suggested that the three stages of leishmania infection are comparable for example to those of syphilis or that they occur in every infection Occasionally some overlapping of the stages may be seen and in such cases it is very difficult to exclude the possibility of double infection with kala-azar and oriental sore. The observations of MIRZORIAN (1941) indicate that a fully evolved visceral infection may become manifest before the disappearance of the primary sore. Our own observations in the Sudan indicate that oro-nasal lesions or even a certain amount of generalized cutaneous infection, can occasionally be found in kala azar patients including cases in which the (untreated) visceral condition appears to be progressing unfavourably. Sometimes as in oriental sore the bodily defences get the upper hand at an early stage, and complete immunity develops with the healing of the primary sore. In kala azar the primary sore is so inconspicuous that special studies have been necessary to demonstrate its existence. In the absence of treatment the majority of kala azar patients succumb to the effects of the visceral infection and therefore never reach the fully developed tertiary stage in which the infection is exclusively confined to the skin and mucous membranes. Kala azar in different places varies in its resistance to treatment and tendency to the development of skin lesions of the post kala azar type while there are apparently many different clinical varieties of purely cutaneous leishmaniasis in the world.

We have suggested (KIRK, 1942) that most of those differences are due primarily to the existence of many different strains of leishmania varying in virulence with different degrees of dermotropic or viscerotropic tendency and some having a special tendency to attack the nasopharyngeal mucous membranes. The marked difference between strains of leishmania causing kala azar and oriental sore is, of course well recognized, but there are in addition minor differences between strains of leishmania, depending on geographical distribution and other factors. The parasites of Indian, Sudan, Mediterranean and Chinese kala azar are not identical in all respects while the Russian workers have shown that there are at least two different types of oriental sore in Turkestan (LATYSHEV and KRIUKOVA, 1942). The biology of leishmania infections provides some basis for the assumption that strains in different regions will tend to vary more or less from each other. Compared with many other infections like syphilis for example the transmission of leishmaniasis does not occur directly from one human being to another but involves an insect intermediary which owing to its delicacy and limited powers of flight is restricted in its range and shows a marked tendency to evolve local species and varieties (THEODOR, 1933). HINDLE (1931) has shown that different strains of leishmania are biologically adapted to local species of the vector and, although there is a general capacity on the part of leishmania to develop into the flagellate stage in various species of *Phlebotomus* it is only when a biological relationship exists between the two that development proceeds further. The influence of additional factors like an animal reservoir has also to be taken into account in certain places as for example, the dog in the Mediterranean

basin, or wild rodents in Soviet Turkestan (LATYSHEV and KRIUKOVA, 1942).

NAPIER and KRISHNAN (1931) have shown that as a result of different degrees of host parasite adaptation leishmania infections may even exhibit differences in clinical features and epidemiology in two localities where the same vector is concerned and no animal reservoir. In Bengal the incidence of post kala-azar dermal leishmaniasis is relatively high, and with the decrease in kala azar incidence there has been a steady increase in the incidence of dermal infections. The infection has now been endemic in Bengal for some generations; consequently when kala azar occurs, even where no treatment is given major epidemics like that of 1854–1873 (Burdwan) do not now arise, and dermal lesions are a much more common sequel of the disease than in the more recently invaded Assam valley where the disease has always exhibited an epidemic character and dermal lesions are uncommon. These authors suggest that the Bengal parasite is undergoing an evolutionary change from visceral to dermal localization whereas the Assam parasite has not travelled so far along this road of evolutionary development. In the latter province, as the general immunity of the population rises through repeated outbreaks of kala azar it is suggested that dermal lesions may be expected to become more common.

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TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

NOVEMBER, 1944.   VOLUME XXXVIII   No. 2

CONTENTS

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TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

THE THIRTY-SEVENTH ANNUAL GENERAL MEETING
of the Society held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 15th June, 1944,

THE PRESIDENT
SIR HAROLD SCOTT, K.C M G   M D   F R.S.E.,
in the Chair

BUSINESS

REPORT OF COUNCIL FOR THE YEAR ENDED 31ST MARCH 1944

The President, before calling on the Hon Secretary to present the Annual Report, referred to various items of special interest during his first year of office. In spite of the difficulties created by the war the Society had progressed and more material had been received for publication in THE TRANSACTIONS, as evidenced by the fact that Volume XXXVII just completed, had eighty seven pages more than Volume XXXVI

He referred to the substantial reduction of the debt on Manson House, and to the hope that before long it would be paid off and that the Society would then be able to utilize more of Manson House   It was with this possibility in view that the Council had decided not to let the upper floors for more than five years

He was glad to note that forty nine new Fellows had been elected during the year

He then spoke of the loss the Society had sustained in the death of nineteen Fellows. He gave briefly details of the careers of some of these, referring particularly to Professor WARRINGTON YORKE, of Liverpool and Dr E C SMITH Local Secretary for Nigeria

The PRESIDENT referred to the work of the Executive Committee, to the Hon. Secretary Dr WENYON whose task had not been rendered any easier by the absence on active service of his fellow Hon Secretary Brigadier FAIRLEY and finally, he made reference to the good work of Miss WENYON and her office staff

The Hon Secretary (Dr WENYON) then presented the Annual Report, which had been circulated to Fellows present at the meeting

Dr NORMAN WHITE proposed the adoption of the Report. This motion was seconded by Brigadier J A. STANTON and carried unanimously.

REPORT OF THE HON TREASURER FOR THE YEAR ENDED 31ST MARCH 1944.

The Hon Treasurer (Dr MARRIOTT) presented his Report together with the Accounts and Balance Sheet prepared by the auditors Messrs. W R. KEEN & Co. and approved by the Audit Committee.

Dr MARRIOTT said the outstanding item was the receipt of the late Mrs M R CALDWELL's legacy of £5 000 which had been applied to further reduction of the debt.

The total cost of Manson House with alterations and furnishing had been nearly £30 000 to meet which it had been originally necessary to borrow from the bank £15,960. By 31st March, 1944 this debt had been reduced to £2,564. He was now glad to announce that at the Council this afternoon it had been decided to utilize for further repayment £1 000 from the Society's General Accumulated Fund. This, together with a few donations received since 1st April, had brought the debt down to £1,559. He hoped the day was not far distant when the debt on Manson House would be fully paid off whether by donations or by legacies.

To return to the Annual Report Fellow subscriptions, sales of TRANSACTIONS and rents, all showed a gratifying increase. Necessary repairs of war damage had been carried out during the summer enabling the vacant parts of the Society's premises to be let for 5 years to suitable tenants. This and the more frequent letting of the lecture hall accounted for the substantial increase under the head of rents received—an item to be still further augmented when a complete year's rent from the Society's tenants was received. Dr MARRIOTT referred to the untiring work of the Secretary Miss WENYON through whose alertness and keen business capacity the new tenants had been obtained. He wished to thank her and her assistants for carrying on so long with smashed windows and no heating in the blitzed building.

Dr STANNUS, in proposing the adoption of the Treasurer's Report, said he was sure everyone was extremely gratified at the present state of the Society.

He recalled the House Committee (of which he was a member) which was responsible, in 1931 for getting the building into running order. He had consistently urged the provision of comfortable seats in the lecture hall. We have them—and he thought this was one reason for the increasing letting of the hall. It seemed quite romantic that the debt was now only £1,559.

Lieut. Colonel DREW seconded the motion which was carried unanimously.

ELECTION OF THE AUDIT COMMITTEE

The PRESIDENT said that Dr A S HODSON and Colonel F P MACKIE wished to retire from the Audit Committee on which they had served for several years. The third member Dr W E COOKE, was eligible for re-election.

Dr C R ASHES and Dr J C BROOM were then elected members of the Audit Committee, and Dr W E COOKE re-elected, for the current year.

This concluded the Annual General Meeting.

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 20th July, 1944, at 3 p m

The President

Sir Harold Scott K.C.M.G , M D F R.S E.,

in the Chair

PAPER

CHOLERA INCIDENCE IN INDIA IN RELATION TO RAINFALL ABSOLUTE HUMIDITY AND PILGRIMAGES INOCULATION OF PILGRIMS AS A PREVENTIVE MEASURE.

BY

Sir LEONARD ROGERS K.C.S I M.D F.R.C.P F.R.S I M S (RET)

In 1926 I published a study of cholera incidence in relation to climatic conditions for India as a whole in 1928 I added data for each province from the time of the earliest vital statistics in 1874 up to 1923 To bring this up to date I have recently studied the data for the further 16 years up to 1939 and the records of such attempts as have been made to put to the test the proposal I had made to control to some extent the frequent spread of the disease by pilgrims (amounting to twenty millions yearly) by inoculating them against the disease before they reached the sacred, but usually insanitary Fairs as they are commonly called. The results (recorded in my Presidential Address to this Society in 1933) of the considerable degree of success which attended forecasts I had published for the 4 years 1930 to 1933 of the probable incidence of cholera in fifteen divisions of India enables the most dangerous times and places of pilgrimages to be foreseen in time to allow preventive inoculations being utilized in this way To enable the data dealt with in the present paper to be followed the principal conclusions of the former papers must first be summarized.

SEASONAL INCIDENCE OF CHOLERA IN INDIA

Chart I in the Memoir ROGERS (1928)—Fig 1 in the Presidental Address, ROGERS (1933)—brings out the striking fact that cholera either dies out, or greatly declines, during the coldest months of the year in all the provinces of India except Assam Bengal and Bihar and Orissa in the north-east, and in south-east Madras. Map III of the Memoir shows that the areas with high winter cholera incidence are just those in which the absolute humidity (which is a measure of combined temperature and humidity) does not fall below 0·400 in January Maps IV to VI and Chart I (loc cit) show that cholera incidence rises successively with the rise of the absolute humidity to over 0 400 in western Bihar in March, in the United Provinces in April and in the Punjab in May This relationship of absolute humidity to cholera incidence has been confirmed in China, the only other large country with endemic cholera. In a similar manner cholera dies down in the early winter months with the fall of the absolute humidity to below the critical point of 0 400

ENDEMIC AND EPIDEMIC AREAS IN RELATION TO ABSOLUTE HUMIDITY

Map VIII of the Memoir (Fig 2 of the Presidential Address) shows that the endemic areas in which cholera never died out completely are precisely those in which the absolute humidity does not fall appreciably below 0·400 in the winter In addition to those already mentioned they include the eastern divisions of the United Provinces and the narrow low lying coastal strip of the Bombay Presidency It is from these that cholera spreads to reinfect the epidemic areas after the complete, or nearly complete, disappearance of the disease at the coldest season of the year The epidemic areas include the western divisions of the United Provinces, the Punjab Sind the major part of the Deccan divisions of the Bombay Presidency and the Central Provinces In all of these the winter absolute humidity falls well below 0 400 in the Punjab it reaches the very low level of 0·250.

ANNUAL VARIATIONS IN THE INCIDENCE OF CHOLERA IN RELATION TO CLIMATIC CONDITIONS.

Diagram II of the Memoir shows the extraordinary variations in the rates per mille of cholera in British India as a whole from 1874 to 1923 Omitting 1874 when the important Bengal data were not available the lowest rates per mille were from 0·30 m 1923 to 0 70 in 1888 the highest were 3 39 3 50 and 3 70 in 1877 1892 and 1900 respectively In the latter years cholera was widely epidemic in most of the provinces of India. In years of high, but not extreme, incidence the epidemics affected much more limited areas only parts of some provinces might show high rates in any particular year I therefore made a close study of forty five divisions, each made up of from three to six districts, usually with populations of one or more million in each. The data of 45 years

for which full records were available were then entered in maps of each year They showed epidemic prevalence on forty-one occasions and brought out the striking fact that no less than forty of them had been preceded by deficiency of the monsoon rains of June to October in the preceding year The one exception was 1894 when the spread of cholera by the 3 000 000 pilgrims attending the 12 yearly exceptionally large Khumbh Fair at Allahabad in a year of unusually high absolute humidity for the season of February was responsible for the epidemic prevalence of cholera in Bihar and the Eastern United Provinces.

Maps of the year of low cholera incidence in 1898 and of very high incidence in 1892 (Maps XII and XI of the Memoir) illustrate important points. In the former the low rate was associated with exceptionally good previous monsoon rains as a whole cholera was absent from the epidemic areas already pointed out. On the contrary in 1892 the previous monsoon rains had been deficient in no less than seven of the eight provinces, as shown in Table I below and famine conditions prevailed.

PILGRIMAGES AND THE SPREAD OF CHOLERA IN INDIA

The cholera epidemic following the Khumbh Fair at Allahabad in 1894 has already been referred to as a matter of fact epidemics due to those 12-yearly large Fairs have occurred regularly from the earliest record in 1882 up to 1930 the last for which data are available In a similar manner the 12 yearly Khumbh Fair at Hardwar where the sacred Ganges debouches from the Himalayas, always spreads cholera over the neighbouring Punjab Further the smaller annual Fairs at Hardwar are recorded to have spread cholera through the pilgrims returning to the Punjab in 17 out of the 22 years between 1900 and 1921 as is illustrated by Chart III in my Memoir Moreover the rate of the spread of the disease is in proportion to the rapidity of communications as shown in Maps XIII to XV In that of 1892 fourteen districts were infected within 10 days of the Bathing Festival this epidemic reached Europe by the overland route in 5 months

The Central Provinces presents a more complicated problem this is illustrated by Map XVI of my Memoir of the incidence in 1906 which demonstrates the invasion of the Central Provinces by cholera carried by pilgrims returning from Puri to the east Allahabad to the north Ujjain State to the north west, Nasik to the west, Pandarpur to the south west and Hyderabad State to the south. Further illustrations of direction of the spread of cholera in India can be obtained from the description in my Memoir of Map IX which shows the frequency of cholera epidemics in each of the forty five divisions of India prepared from the forty five yearly maps already mentioned. It clearly shows that the Central Provinces is most frequently invaded from both the east and the west contrary to BRYDEN's wind borne theory of the seventies of the nineteenth century

An Analysis of Years of High and of Low Cholera Incidence in the Six Decades 1880 to 1939

In the light of the foregoing considerations I can now turn to an analysis of the main factors influencing the incidence of cholera in India during the last six decades—as shown in Table I of this paper. Average data for British India as a whole and for the eight main provinces are given at the top for purposes of comparison. Below the average rates per mille of each decade the data of the years of exceptionally high and exceptionally low incidence in India as a whole are entered. the high rates both for India and for each province are printed in black figures to emphasise them. The years in which the previous monsoon rains were deficient are shown against each province by a − sign. likewise years of exceptionally good rains are indicated by a + normal years are left blank. Under the United Provinces in the column "Pilgrim Fairs," Allahabad Khumbh Fairs are marked AK. likewise, Hardwar Knmbb Fair years are indicated by HK. As the average figures for each decade are most influenced by the exceptionally high and low incidence years, a bird's-eye view of the whole period is obtained and an analysis will permit of conclusions as to how far the yearly variations are explained by climatic conditions, together with the effects of the larger pilgrimages.

1880-89 —The average rate for India was close to that of the six decades. 1880 showed low cholera. The previous rains had been normal to good except in the United Provinces there low rainfall was followed by a higher cholera rate than the average for the decade the only province to show this. Likewise 1881 had low cholera after exceptionally good rains in three provinces. 1882 had a high cholera rate—it was highest in Assam—after low rainfall. High rates in Bengal Bihar and the United Provinces, in spite of average previous rains, are explained by the spread of cholera by the millions of pilgrims attending the Allahabad Khumbh Fair of this year. Cholera was high in 1885 1887 and 1889 in each case rainfall had been deficient in three or four provinces, and in each year the highest provincial cholera rates occurred in provinces with previous deficient rainfall.

1890-99 —The average India rate for this decade is higher than that of the whole series. The reason is not far to seek five out of the first 7 years had cholera rates above that of the decade. With the exception of 1894 the high rates were once more associated with previous low rainfall in from three to six of the eight provinces. In 1892 in addition the Hardwar Khumbh Fair was responsible for high rates in the Punjab and the Western United Provinces this was the famine year already referred to. On the other hand, 1898 and 1899 showed exceptionally low cholera rates following exceptional good rainfall—in accordance with the general rule.

1894 is the exceptional year already mentioned in this good previous rains were followed by very high cholera incidence. The highest rates were in Bihar and the adjacent eastern divisions of the United Provinces both resulted

TABLE I

CHOLERA ANNUAL DEATH RATES PER MILLE IN BRITISH INDIA.

	British India		Assam		Bengal		Bihar Orissa	United Provinces			Punjab	Central Provinces		Bombay		Madras	
Millions population			–		46½		34	43			20½	14		19		41	
Rainfall Average			100 m.		5 in		34 in.	38 in			20 in.	40 in		45 in.		32 in	
Deaths	189 309		300		62,340		50 433	27 418			1 807	11 5 7		0 008		25 042	
Ditto per mille	1 44		0 96		1 45		1 20	0 56			0 11	0 73		0 4		0 58	
Year	Deaths per Mille	Total Deaths	Deaths per Mille	Previous Rains	Deaths per Mille	Previous Rains	Deaths per Mille / Previous Rains	Deaths per Mille	Previous Rains	Pilgrim Fairs	Deaths per Mille / Previous Rains	Deaths per Mille	Previous Rains	Deaths per Mille	Previous Rains	Deaths per Mille	Previous Rains
1879–89	1 50	201 800	2 69	1 89		2 34 –	1 38	0 19	1 81	1 0	1 3 –
1880	0 64	113 532	0 4	0 69		0 74 +	1 07 –	0 01	0 03	0 04	0 02
1881	0 87	159 188	1 12 +	1 3 +		1 28 +	0 84	0 30	1 10	1 01	0 3
1882	1 79	34 031	4 69 –	2 79		3 33	2 02	Ak 0 00	1 33	0 48	0 8
1885	1 91	3 5 60	1 71	3 67 –		3 54 –	1 44	0 10	2 23	2 27 –	2 0 –
1887	2 45	473 830	1 75	1 79		4 31 –	4 54 –	0 47	2 36	1 36	1 01
1889	3 15	415 988	4 04 –	2 09		3 86 –	1 09 +	0 15	5 55 –	1 97 –	2 7
1890–99	1 89	398 836	4 71 –	2 63		2 44 –	1 67	0 18	* 13	1 25	1 86
1891	2 51	53,409	4 70 –	3 50		2 97 –	3 63 Hk	0 41	2 37	0 85	3 5 –
1892	3 40	7 4,334	4 29	2 99 –		4 60 –	4 15 –	2 70 –	3 40	2 28 –	2 3 –
1894	2 44	571 64	2 67	2 42 +		4 48 +	3 80 + Ak	0 003 +	0 83 –	1 73 –	1 3
1896	2 31	489 6 9	3 31	3 20 –		3 25	1 4	0 25	6 29 –	1 88 –	1 40
1897	2 59	550 87	6 63 –	*94		2 61 –	0 94	0 03	5 53 –	3 03 –	4 38 –
1898	0 71	181 310	2 22 +	1 44		0 60 +	0 05	0 002	0 0	0 23	2 0 –
1899	0 79	1 1, 8	1 66	1 8		1 12 +	0 47	0 09	0 05	0 40	0 9 +
1900–09	1 91	41 06	2 01	2 80		3 14	1 30	0 32	1 90	1 53	1 77
1900	3 72	70 * 3	4 51 –	3 6*		6 46 –	1 81 –	1 37 –	6 60 –	3 71 –	1 80
1904	3 05	192,835	1 08 +	2 41		1 09	0 14	0 04	0 24	0 1	0 5 +
1906	3 05	690 541	6 38 –	3 23 –		4 08 –	3 14 – Ak	0 21	3 26	2 60 –	3 9 –
1908	2 61	591 23	4 22 –	*94 –		5 63 –	1 75 –	0 81	0 75	0 09	3 9 –
1910–19	1 63	386 319	3 09 –	63		2 89	1 44	0 22	1 37 –	0 99	1 52
1911	1 12	26 00*	1 81 +	0 99 +		3 1 –	0 46 +	0 07 +	0 03 +	0 8	1 6 –
1918	2 35	560 80*	2 32	1 8		5 9 –	2 56 Ak	0 01 +	0 74 +	0 45	3 0 –
1910	– 43	5 84*6	5 61 –	2 8 –		3 0 –	1 74 –	0 44	4 46 –	2 63 –	2 3 +
1920–29	0 94	227 438	1 47	1 5		1 43	0 81	0 74	0 81	0 35	0 9
1921	1 87	4 50 608	1 8*	1 7		2 6	3 39 –	0 94 –	4 19 –	0 18	1 87
1923	0 30	78,002	0 54 +	0 9 +		0 2 +	0 6 +	0 001	0 18	0 43	0 1 +
1925	1 45	351 803	1 01	2 9 –		2 2 –	0 99	0 10	0 88	0 36	1 4
1930–39	0 63	10 512	0 63	1 08		1 11	0 50	0 04	0 91	0 45	0 39
1930	1 40	332,222	0 9 –	1 *0		4 50	1 35 Ak	0 06	1 67	0 0	0 46
193	0 74	67 *19	0 6	0		0 2	0 2	0 02	0 03 +	0 06 +	0 1 +
1933	0 23	69 318	0 7	0 6		0 5 +	0 4	0 003	0 1	0 4	0 1 +
1935	0 86	226 143	1 33 –	1 4		0 4	1 3 Hk	0 19	0 1	0 1 +	0 1 +
1939	0 33	9 568	0 4 +	0 6		0 5	0 5 +	0 0	0 1	0 1 +	0 04

Ak = Allahabad Khumbh Fair   HK = Hardwar Khumbh Fair

from the spread of cholera by the 3 000 000 pilgrims returning long distances through that endemic area from the Allahabad Khumbh Fair   The higher incidence in those provinces than in the Khumbh Fair of 1882 I traced to the absolute humidity being unusually low and unfavourable to the spread of cholera in 1882, but in 1894 unusually high for the season of the year and favourable to epidemic incidence   It will be noted that the total cholera death rate for India exceeded 500 000 in 3 years of this decade and that in the famine year 1892, it nearly reached three-quarters of a million   in that year failure of the preceding winter rains aggravated the effects of the preceding exceptionally low monsoon rain in no fewer than six of the eight provinces   The high average cholera incidence in this decade is therefore fully explained by failures of the rains and the spread of cholera infection by Khumbh Fairs.

1900-09 —The average rate was again at a high level   namely 1·91 per mille   There were only 3 years of high incidence but they include the record incidence of 797,273 deaths, 3 72 per mille in 1900   another famine year with previous deficient monsoon rains in six provinces followed by low winter rains 1908 and 1909, with almost 600 000 and 700 000 cholera deaths also showed previous deficient rains in five and six provinces respectively   In 1906 the incidence was increased in Bihar and the United Provinces once more by the occurrence of another Allahabad Khumbh Fair   In this decade again it will be observed that the highest provincial death rates nearly always followed deficient rain in the particular province.

1910-19 —The average cholera rate in this decade varied little from that of the whole period under consideration   In 7 years the rate did not vary materially from the average rate   1917 had a low rate following particularly good rains in five provinces   1919 showed the highest cholera rate following deficient rainfall in six provinces   the number once more exceeded half a million deaths   In 1918 the Allahabad Khumbh Fair was yet again responsible for a high total rate with the highest provincial ones in the Bihar and the Eastern United Provinces   in spite of normal previous rains in those areas   It is noteworthy that the only other high provincial rate was in Madras following deficient rainfall in that province alone   The low moderate incidence in this decade of 1·63 is explained by the fact that it contained only 2 years of exceptionally high incidence   in neither of these was the half a million rate seen.

1920-29 —The average cholera incidence of 0·94 per mille in this decade is considerably below that of the whole series of years   No year showed an extremely high rate   the highest were in 1921 and 1923 with very low rainfalls in four provinces in the former with a rate of 1 87   and in two provinces only in 1928 with a rate per mille of 1 45   On the other hand 1923 showed the lowest recorded cholera mortality, up to that date of 0 30 per mille   It is significant that the previous rains had been exceptionally good in no fewer than five of the eight provinces and up to the normal in the remaining three   The low average rate for India as a whole in this decade is readily explained by the climatic

conditions having been exceptionally unfavourable to the development of serious cholera epidemics this was aided by the absence in this decade alone of any Allahabad Khumbh Fair to produce a serious epidemic of cholera

1930–39 —The still lower average cholera death rate of 0 65 in this last decade, following a comparatively low rate in the previous decade, raises the interesting question whether a lasting reduction of cholera is gradually being brought about through improved sanitary measures The highest mortality of 337,322 was in 1930 in spite of previous good rains As usual it was mainly in Bihar and the United Provinces, with an extension to the Central Provinces as the result of infection carried by the Allahabad Khumbh Fair I shall have to return to this event presently Likewise the next highest mortality was in 1938 it was due to high rates in the Punjab and the west of the United Provinces as the result of cholera spread by pilgrims together with a high rate for the decade in Assam, which was the only province with previous low rainfall Neither outbreak was as severe as in some of the years already considered with deficient rainfall in several provinces

On the other hand, this decade alone included 3 years of record low cholera mortality in each of them the previous rains were very good and in excess of the normal in two or three provinces namely in 1932, 1933 and 1939 In the first two of these I had actually forecast low cholera incidence on the rainfall data up to October of the previous year

It must therefore be reluctantly admitted that the exceptionally very low incidence of cholera in this decade was essentially due to the absence of a single year in which the climatic conditions favoured epidemic prevalence of cholera such as had occurred in every previous decade.

That is not to say that no benefits have accrued from sanitary progress in India during the last few decades There is evidence that cholera incidence has been reduced in cities and large towns through the provision of modern water supplies and that where it has been possible to improve the sanitation at the sites of pilgrim Fairs outbreaks of cholera at the Fairs themselves have been reduced Recent health reports in India have however recognized the truth of my contention that improved sanitation at the Fairs cannot by itself prevent cholera infection of the pilgrims in the course of their long journeys, commonly of several hundred miles through insanitary areas which are usually infected in the endemic areas and may become epidemic ones whenever the absolute humidity is high and especially when the previous rainfall has been deficient. That in its turn is due to the fact that about 90 per cent. of the population of India live in villages, in very few of which good water supplies have yet been made available although great efforts are now being made to remedy this state of affairs as far as the poverty of the people and the necessarily low taxation limits permit

Moreover in towns with many well qualified doctors, the system of treatment worked out by the writer of giving large quantities of hypertonic and

alkaline salines intravenously saves many from succumbing to cholera. Unfortunately efficient treatment is not practicable in the vast number of small villages, in which the great majority of the population of India reside, for want of medical practitioners versed in the European system of medicine and of hospitals and other facilities for carrying out the modern treatment of cholera.

Lessons to be Derived from Epidemiological Studies of Cholera.

The foregoing analysis suffices to prove that an examination of the cholera data of the last 16 available years amply confirms the conclusions arrived at in my earlier papers on the subject. The main lessons are —

1. A close watch on the June to October south west monsoon rains enables high cholera incidence to be foreseen in the autumn months in the endemic areas with absolute humidities always over 0.400 and several months before the spread of epidemics of cholera in the next spring from the endemic to the epidemic areas.

2. The danger of cholera being spread by the return of pilgrims from any particular Fair can also be foreseen from the climatic data at the time and knowledge that cholera is present in the areas through which the pilgrims have to travel. This will be illustrated further in the following account of the Pandarpur Pilgrimage.

3. As the climatic conditions are beyond human control the only practical method of controlling and limiting the spread of cholera by the many millions of pilgrims every year is by immunizing them against the disease by preventive inoculation preferably before they reach the Fairs.

Recent Progress in the Use of Preventive Inoculation of Pilgrims.

In the year following the publication of my first paper on cholera epidemiology a 12 yearly very large Khumbh Fair was due to be held at Hardwar in April 1927. I was sanguine enough to hope that the able Public Health Commissioner of the Punjab would see his way to arrange for the Punjab pilgrims to be inoculated before starting on their perilous journey with a view to controlling the otherwise inevitable widespread epidemic in his province. Unfortunately he decided that it was not practicable to do so: the epidemic duly took place with the highest provincial death rate from cholera since the preceding Hardwar Khumbh of 1915. Once more, in 1938 the Hardwar Khumbh Fair on April 13th was responsible for infection of cholera being carried by the returning pilgrims to every district in the Punjab by April 29th a little over a fortnight. The deaths from cholera in 1938 totalled 5,780 almost ten times the average of the previous 5 years without any Khumbh Hardwar Fair. The health report for 1938 admits that Khumbh Fairs at Hardwar "are invariably accompanied or followed by severe outbreaks in the Punjab and 1938 has been no exception to the general rule. No measures up to the present time have been effective in preventing outbreaks which were

anticipated. General hygienic measures were used, they did not include to any great extent protecting the individual against the disease Inoculation was not considered to be a practicable procedure as it was not acceptable to the people. Hope, however was expressed that fuller knowledge may be available before the next Hardwar Khumbh Mela that is after another 12 years

In 1930 the far more dangerous Allahabad Khumbh Fair was due to take place in January and February My position at the India Office at the time enabled me to draw up a memorandum on the serious epidemics of cholera that had occurred at every such 12 yearly Fair of which vital statistics are available from 1882 to 1918 (See pp 123-130 of my Memoir of 1928) This was forwarded by the India Office to the authorities in India and duly considered by them they decided that in the political conditions of the time compulsory inoculation of the three million pilgrims expected at the Fair was impracticable the anticipated epidemic followed with 147 000 deaths from cholera that year in Bihar nearly 60 000 in one month together with 30 000 deaths in the neighbouring eastern divisions of the United Provinces.

The authorities on the spot were doubtless right in their opinion and epidemic spread of cholera by the Allahabad pilgrims must presumably be accounted as one of the blessings of democracy prematurely granted to a country only about one tenth of whose population can read or write I must confess to a reactionary preference for the autocratic, but effective, action taken by Sir RICHARD TEMPLE in 1857 when he permanently prohibited the holding of the cholera spreading pilgrimage to the Parchman Hills and ordered other Central Provinces pilgrimages to be held during winter months when cholera epidemics do not occur as we now know on account of the low absolute humidity at that season.

In the *Public Health Reports* for 1930 and 1931 of the United Provinces and of Bihar an attempt was made to deny the spread of cholera by the three million Khumbh Fair pilgrims in 1930 on the grounds that there was little cholera at the Fair itself and that the terrible North Bihar epidemic did not follow immediately on the Fair That assumption ignores the facts (1) that the great factor in spreading cholera is the passage of the pilgrims through innumerable insanitary small towns and villages in a huge endemic area from which cholera is never absent in the winter in a sporadic form although this contention of mine has repeatedly been acknowledged in recent health reports of India as correct. (2) The absolute humidity is too low in February for epidemic cholera to occur in the regions affected by the epidemic the seeds of this are then sown by the pilgrims, the harvest is reaped in due course when the absolute humidity rises sufficiently high in March and April Nor is any other explanation given of the invariable cholera epidemics following the Allahabad Khumbh Fairs and that too in years when the previous rains have been good and unfavourable to cholera epidemics as in 1882, 1894 1918 and also in 1930 itself

Inoculation on a voluntary basis was available but little used   in short every
thing possible was done short of compulsory inoculation of the pilgrims. The
results are shown in Table II

Table 11 shows the total deaths and the rate per mille in the Bombay
Presidency the deaths in the first and second halves of the year in the Sholapur
District, the number of pilgrims at the July Fair the number compulsorily
inoculated in the last 6 years, 1936 to 1941 the cholera cases and deaths during
the Fair and the spread of cholera by the returning pilgrims.

1930 —Cholera widely epidemic in the second half of the year   15 124
deaths. No cholera in Sholapur district in May and June   it broke out during
the July Fair among pilgrims from the cholera infected Central Provinces and
Hyderabad State   spread by pilgrims over neighbouring Deccan districts and
continued up to December

1931 —Severe epidemic with 18,616 deaths. Sholapur district free before
the Fair but cholera prevalent in the adjacent Southern Deccan in the first
6 months of the year   Cholera broke out among the pilgrims during the Fair
with 107 cases in hospital and thirty-eight deaths. Widespread epidemic due
to the pilgrims in the Deccan and carried by them to the Central Provinces.

1932.—Record low cholera year in India after good previous rains. Only
one cholera death during the first 10 months of the year in the Sholapur dis-
trict   consequently no spread of the disease by the 135,200 Pandarpur pilgrims—
a very exceptional event.

1933 —Moderate cholera incidence in the province as a whole with 7 797
deaths. Cholera had broken out in the Sholapur district in November 1932,
and January to June, 1933 showed 455 deaths in the district   the highest of
the 12 years in the table   It increased during the July Pandarpur Fair in the
Sholapur and adjacent Deccan districts and was widely prevalent to December
The disease had been brought to Pandarpur by the July pilgrims with 173 cases
and eighty three deaths during the Fair   There had also been an increase after
the April Pandarpur Fair   The annual health report states that these Fairs
were responsible for the severity of the epidemic in the Deccan, which was
most severe in the central districts adjoining the Sholapur district. The Central
Provinces report records that the Pandarpur pilgrims also spread the disease
to the western division bordering on the Bombay Deccan

1934.—Cholera widespread especially in the Northern Deccan   11,362
deaths. No cholera deaths in the Sholapur district in the 4 months up to June.
After the Pandarpur Fair in the middle of July cholera moderately prevalent in
the Sholapur district to the end of the year but no spread by the pilgrims
recorded   The previous rains had been good and unfavourable for cholera
epidemics.

1935 —Total cholera deaths in the province 11,235   Cholera epidemic in
central and southern Deccan and low in the northern districts after high incidence
there the year before. The disease was m   \   alent during the first

half of the year in the Sholapur district following the Pandarpur Fair early in July the disease became epidemic in this and in the surrounding districts of the Deccan

We thus see that in 1932 in the absence of cholera in the Sholapur district there was naturally no spread of the disease by the pilgrims in 1934 with no cholera in the district before the Fair and good previous rains cholera was only moderately prevalent in the Deccan after the July Fair In the other 4 years extensive epidemics followed the July Fair in 2 of those years the pilgrims also carried the disease to the adjacent western Central Provinces

The Incidence and Spread of Cholera after the Introduction of Compulsory Inoculation of Pilgrims in 1936

This year proved a crucial one in the prolonged attempts to control the spread of cholera by the returning Pandarpur pilgrims by public health measures

The 11,312 deaths from cholera recorded in 1936 nearly all occurred in the Deccan districts of the Bombay Presidency the northern and coastal districts were only lightly affected. The Sholapur district was practically free during the first 5 months of the year but it became severely infected in June with 345 deaths, immediately before the Pandarpur Fair principal day due on 30th June. Many of the pilgrims came from the Central Provinces and the Hyderabad State both had already become severely infected by pilgrims returning from the Allahabad and other Fairs and the pilgrims had to travel through the heavily infected areas of the Sholapur district to reach Pandarpur The danger was much enhanced by the previous monsoon rains having been deficient in the Bombay Deccan. Thus everything pointed to serious diffusion of cholera by the pilgrims attending the Ashadi Fair at Pandarpur

To meet this grave emergency on 20th June the Bombay Government courageously issued a notification under the Epidemic Diseases Act to prohibit any pilgrims from entering Pandarpur during the period of the Fair (which extends over a number of days) unless they could produce evidence of having been inoculated against cholera within the preceding 3 months Arrangements were made to supply free inoculation at railway stations and at each halting place of the bands of pilgrims through the accompanying medical officers The response of the pilgrims on the whole was good but some opposition was met with in this, the first year of the trial of this drastic measure

The data in Table II show that over 90 per cent of the pilgrims were inoculated during their journey to Pandarpur most of the remainder were reported to have brought with them certificates of recent inoculation More over 11 752 of the permanent inhabitants of Pandarpur town out of a population of 29 000, were inoculated. The admission to the isolation hospital during the Fair of forty-eight cholera cases with twenty two deaths was a further indication of the danger of the pilgrims spreading the disease during their return journey had they not been inoculated. However carefully collected statistics of cholera

The Present Position of Compulsory Inoculation of Pilgrims in India.

The following information has been gleaned from the Reports of the Public Health Commissioner with the Government of India and of the Central Advisory Board of Health for 1939 and 1940 more particularly

Provincial Health Committee Reports of 1913 to 1916 made many recommendations for improving the sanitary conditions of pilgrim centres They reported that their control was very much more effective than it had been 30 years before but control of the Fairs is insufficient owing to the long distances travelled by the pilgrims through insanitary towns and villages. They state that " It is interesting to note that none of the Pilgrim Committee Reports makes any reference to the protection conferred by inoculation with anti-cholera vaccine. Further the suggestion of Sir Leonard Rogers to inoculate the pilgrims going to the Allahabad Khumbh Fair of 1930 was referred to by all the provincial Directors of Public Health it was unanimously rejected " as impracticable inexpedient and even dangerous. All of them, however accepted the evidence that the vaccine provided valuable protection against infection and they advised strenuous efforts to persuade the people to submit to inoculation by every possible measure that is short of using any form of compulsion.

The disastrous spread of epidemic cholera by the 1938 Khumbh Fair over the Punjab and other provinces, already mentioned, caused the Punjab health authorities to raise the question of compulsory inoculation of pilgrims once more In the meantime the success of that measure in the case of the Pandarpur Fair during 1936 to 1939 had been reported. These events led the Central Advisory Board of Health in 1939 to appoint a committee to consider the adoption of compulsory inoculation at other Fairs in 1940 their recommendation to carry out trials at pilgrim Fairs in every province of India was endorsed by the Board of Health. An Indian member pointed out that in the performance of their religious duties the pilgrims must not endanger the lives of other people through spreading cholera infection as the result of refusing to be protected by inoculation The recent establishment of the identifying characters of the true cholera vibrio enhances the present value of the vaccine as stressed by Major General Bradfield this makes the present time singularly opportune for the extension of the use of the vaccine. Moreover the increased efforts of the last few years to popularize its use has helped to educate the people regarding its valuable protective properties. Thus, the average total annual recorded inoculations against cholera in British India has risen from 2,700,000 in 1928-33 to 6,649,000 in 1934-39 more than a two-fold increase

The use of compulsory inoculation of pilgrims in India has its limits it cannot be expected altogether to prevent serious epidemics in future years when the spread of cholera is favoured by climatic conditions. Nor can it prevent the continued yearly occurrence of the disease in its endemic areas.

Nevertheless 6 years experience of compulsory inoculation of Pandarpur pilgrims in the Bombay Presidency indicates that its use can do something to lessen the incidence of cholera in India—so largely due to the spread of the disease from the endemic to the epidemic areas—*far more effectively* than the sanitary measures at the Fairs themselves hitherto relied on Further trials will be awaited with interest. I do not, however, expect that the immediate saving of life will be so great as further to embarrass those who are so greatly concerned by the over population of India

CONCLUSIONS

The lessons to be derived from my prolonged studies of the epidemiology of cholera in relation to climatic conditions have already been stated (See p 80)

The only conclusion to be derived from the foregoing discussion of the use of compulsory inoculation of pilgrims with a view to reducing the spread of cholera by them is that the recorded success of 6 years trial at Pandarpur fully justifies the further trials of the plan at pilgrim centres in other provinces of India as already recommended by the *Central Board of Health.*

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DISCUSSION

Colonel C A Gill Mr President and gentlemen there is no one entitled to speak with greater authority on the subject of cholera than Sir LEONARD ROGERS, and I feel sure that the interesting lecture to which we have just listened will be scanned very eagerly by the public health authorities not only in India but in other countries where cholera prevails Sir LEONARD ROGERS divides his paper more or less into two parts In one part he deals with certain aspects of the epidemiology of cholera, and in the other with the compulsory inoculation of pilgrims against cholera. I should very much like to deal with the first part, but the Chairman has warned us that speeches must not exceed 10 minutes, and so I will confine my remarks to the question of the compulsory inoculation of pilgrims attending religious fairs in India. I have a particular reason for doing so because I was Director of Public Health in the Punjab at the time when Sir LEONARD, as he has just told us, made strong representations on the subject. By implication he rather suggested that, to put it mildly we

were not very forthcoming. It is, however, one thing to make a suggestion, which may be a very good suggestion in theory, but to render it practicable it must be adapted to local conditions. Sir LEONARD was then at the India Office and I do not think he quite understood the practical difficulties, otherwise he would perhaps have not been quite so strong in his remarks in regard to our unhelpful attitude. But let me make two points clear before I explain the difficulties. The first is that cholera is the bane of the life of the Public Health Departments of India, and as Director of Public Health in the Punjab, I would have been delighted to adopt any measure however drastic and however difficult if it promised to relieve me of some of the anxiety I went through every year especially those in which big fairs are held at Hardwar on account of cholera. That is the first point. The second is that one naturally takes great notice of anything emanating from Sir LEONARD ROGERS, and consequently one would give very careful consideration to any suggestion coming from him. Apart from this I was under a personal obligation to Sir LEONARD ROGERS and would do anything possible to forward any scheme he put forward. For many years it was my duty to study epidemics, mostly malaria, in the Punjab. I held a post which had to be renewed every year and every year when there was no epidemic the Government said, What is the use of keeping this man? There is nothing for him to do. If there was an epidemic it was said. What is the good of this officer if he had been any use there would have been no epidemic." I was thus frequently in danger of being officially liquidated, but on two or three occasions, without my knowledge or suggestion, Sir LEONARD ROGERS wrote to the Government and got the appointment extended. On all grounds, therefore I should be the last person to put any difficulties in the way of introducing the compulsory inoculation of pilgrims against cholera, providing it was practicable and would be reasonably successful. Unfortunately I was not satisfied that either of these promises were fulfilled. I studied many cholera outbreaks in the Punjab over a period of 8 or 10 years, and found in almost every instance they were due originally to infection imported, directly or indirectly from Hardwar, for as Sir LEONARD says, cholera is not endemic in the Punjab. Enquiries made while the facts were fresh show that in quite a considerable number of cases the pilgrim who brought the infection from Hardwar did not suffer himself from cholera, but that 2 or 3 days after his arrival cholera broke out among members of his family. This would have been puzzling were it not for the fact that it is the invariable custom of pilgrims to bring back from Hardwar a bottle of water from the Sacred Pool. Now it not infrequently happens that persons suffering from cholera are drowned in the Sacred Pool, but apart from this, the pool is liable to be heavily contaminated by the innumerable bathers. It has never been proved that cholera is imported into the Punjab by their bottles of water, but it seems highly probable that infection is sometimes imported in this manner or by sweetmeats, for it is the custom of pilgrims on their return from

Hardwar to call together their friends and relatives who have not had the opportunity of going to the Fair to give them some of the sacred water It is, I suppose, a vicarious way of doing the pilgrimage In one instance in which the pilgrim did not get cholera but one of his relatives did, I found that this man was indirectly responsible for the infection of fifteen villages and 325 deaths from cholera It is thus clear in view of the habits and customs prevailing in the Punjab that the inoculation of the pilgrim alone is not going to prevent the importation of infection which, once introduced is apt to spread with alarming rapidity This constitutes one loop-hole but there is another I am very much surprised that Sir LEONARD had nothing to say about the value of cholera inoculation. I have not seen any recent reports so I do not know what the present position is, but at the time of which I am speaking cholera inoculation gave no sort of solid immunity It is very difficult to obtain reliable data for statistical analysis but I think most people with first hand knowledge agreed that the official cholera vaccine in use about 10 years ago afforded a strictly limited degree of protection. I may perhaps be a little prejudiced because I suffered from a very severe attack of cholera in 1919 in spite of the fact that I took every precaution and had been inoculated regularly every 3 months for nearly 2 years previously Here then is another loop-hole and when we were urged to enforce a naturally unpopular measure when success could not be guaranteed, there was naturally some hesitation The political implications of compulsory inoculation at this time were important. It might be different in Pandarpur people there might accept compulsory measures but people in the Punjab are apt to react violently to unpopular measures. The Punjab Government had an unfortunate experience with compulsory plague inoculation In the early days of plague they tried to stop plague by making plague inoculation compulsory but there were riots and bloodshed, and some public health doctors lost their lives. I do not think that would happen in the south of India, but Governments have to take into account local conditions These, then, were the reasons that rendered it necessary to turn down Sir LEONARD s proposal which I would otherwise have been most willing to support. The Punjab Government said, in effect, If we adopt Sir LEONARD ROGERS' proposal will you guarantee that there will be no serious outbreak of cholera in the Punjab ? and as I did not feel justified in giving any guarantee, they were not prepared to take the political risk, and I think they were right. Although we were not able to make cholera inoculation compulsory Sir LEONARD s suggestion was most helpful, because the Punjab Government, having turned down this proposal which had been given a great deal of publicity felt bound to redouble their efforts in other directions to minimize the danger from cholera. Consequently I was enabled to obtain staff and equipment for dealing with cholera epidemics on a scale previously unprecedented and therefore once more I am under an obligation to Sir LEONARD

But all this is ancient history I believe now that almost all opposition to preventive inoculations has vanished in the Punjab At any rate on one occasion before I left the Punjab there was a riot in the plague infected village because the doctor ran out of anti plague vaccine—and the whole village could not be *voluntarily* inoculated on the same day ! It may therefore well be that the adoption of compulsory inoculation against cholera is now practicable in the Punjab and if as I understand is the case, the vaccine now in use gives a greater measure of protection than it did 10 years ago the compulsory inoculation of pilgrims might help to solve the terribly difficult problem of cholera at the great religious fairs of India.

Lieut -Colonel S P James   Colonel GILL mentioned that anti-cholera inoculation had not protected him against an attack of the disease and as I had the same experience in Mesopotamia in 1916 I should like to say a few words. It may be true as I think Colonel GILL said that the vaccine now available is more effective than the material with which HAFFKINE made the first anti-cholera inoculations in Bengal in 1896 or at any rate that it is more effective than that used on British troops in the last war   I was sorry that Sir LEONARD s paper did not include information on this subject Assuming, however that anti-cholera inoculation does really provide protection, I suppose that most people would be prepared to agree that on the results of the trials at Pandarpur further trials at other pilgrim centres might be justifiable.

But, even if further successes at those centres were obtained, I think it would be unfortunate if they were to lead to a widespread adoption of anti-cholera inoculation as the method of choice for trying to reduce the incidence of cholera in India generally   Undoubtedly the measure would be justifiable as an emergency plan for protecting particular groups or assemblies of people, but it should not take first place in the long term programme of sanitary reform on which everyone hopes India is engaged   Cholera is the best example of a tropical epidemic disease which is amenable to improvements in environmental, domestic, and personal sanitation. Everyone agrees that India is greatly in need of improvement in those respects as well as in getting more and better food more and better education, and a higher standard of living generally Indeed, Sir LEONARD himself has a short paragraph in his paper in which he raises the question whether a lasting reduction of cholera is already being brought about in British India through improved sanitary measures. I wish he would make a detailed analysis of statistics on that subject. The charts of mortality from cholera which he showed, contain statistics which should not be lumped together in an enquiry to ascertain the effect of sanitary measures in reducing cholera. In those charts the statistics of places in which there had been no sanitary improvement, swamp the decrease of cholera in places where, for example, there has been an improvement such as the provision of a satisfactory reasonably safe water supply The decrease of cholera in those places is a notable achievement of sanitary effort in India.

Dr F C Collingwood As Medical Officer of the British Overseas Airways Corporation, I am intensely interested in this subject of the spread of cholera, not so much amongst pilgrims as its spread on an international scale by passengers and crews travelling by air

My late Chief Medical Officer Colonel F P Mackie, whose sudden death last week is lamented by many members of this Society had hoped to he present at this meeting and was, I believe intending to make a few remarks on the question of cholera and air travel

I am not in a position to offer this meeting anything in the way of useful comment, but I would like to take the opportunity of asking Sir Leonard's advice in regard to any special precautions that an international air lines organization should take to limit the spread of cholera in particular would he advise that crews be maintained in a state of immunity when passing regularly through endemic areas, or should inoculation only be given when cholera is actually present or is likely to occur ?

We have, of course, to comply with any regulations laid down by the health authorities of other neighbouring countries particularly as concerning aircraft arriving in Egypt from India

If there is anything in this connection that Sir Leonard would like to advise which might be of interest to this meeting I should be very grateful

Sir Leonard Rogers, in reply said I am greatly interested in the observations of my friend Colonel Gill, whose epidemiological studies are well known I sympathize with him in his difficulties over trying compulsory inoculations of pilgrims in the Punjab with its fanatical people, for in 1901 I had to face a difficult position when sanitary officer at a Puri pilgrimage. Sporadic cases of cholera were occurring and those dying of cholera were being placed on the steps of the sacred tanks so that their stools must have contaminated the water which was being taken away by the pilgrims to their camping grounds with the certainty of a severe epidemic resulting as it did in 1912, under similar conditions The I C S District Officer had the courage to adopt my suggestion that he should put guards on the tanks and empty the lotas of water although we well knew that a riot might occur in which if we escaped having our heads broken, we should certainly be broken by the Bengal Government Fortunately all went well and a serious epidemic was averted Colonel Gill was I think wise in not taking such a risk in the Punjab but I was careful in my paper to say that the question of using compulsory inoculation of pilgrims was one for the provincial administrative and sanitary authorities to decide on the spot, and I blamed the political situation, and not the public health officers, for the failure to utilize this weapon. With regard to the efficacy of anti-cholera inoculation, I agreed that formerly it was not always fully effective, but now we know the true characters of the cholera vibrio it is likely to be more so I also mentioned that recently all the provincial Directors of Public Health had agreed that the method is effective so I did not deal with that point, partly for want

of time. The most conclusive proof of the efficacy of anti-cholera inoculation I know of was recorded by Dr C. A. BENTLEY in Bengal some years ago. A very large village in north Bengal was attacked with cholera   about half the population were Hindus and the rest Mohammedans. Voluntary inoculation was provided, but only the Hindus accepted it. After about a week the epidemic ceased among the Hindus but continued among the Mohammedans, whose male members only then accepted inoculations. After about another week Mohammedan males ceased to be infected, but their women living in the same houses, continued to be attacked. They then submitted to be inoculated and the plague ceased [1] The large scale inoculation of pilgrims from India and the Dutch East Indies to Mecca over several decades is also generally accepted as having played an important part in the prevention of the spread of the disease to Europe in recent times   I am very glad to hear that Colonel GILL thinks that the time may now be ripe to use compulsory inoculation of pilgrims more freely for that is the main point of my paper and his successor in the Punjab appears to have come to the same conclusion, but only after witnessing the disastrous effects of its neglect at the time of last Hardwar Kumbh Fair in 1938.

Colonel JAMES urges that the pressing of sanitary improvement in India to get to the root of the matter should not be neglected in favour of compulsory inoculation of pilgrims. So far from making any such suggestion in my paper I stressed the importance of the increasing but necessarily very slow progress in that direction in India at the present time

In reply to Dr COLLINGWOOD regarding prophylactic inoculation of air plane travellers in the Indian route, I think their inoculation should be confined to those going to or from areas in which cholera is epidemic, which can now largely be foreseen as shown in the first part of my paper It only remains for me to thank you all for the patient hearing you have given me

COMMUNICATIONS

MALARIA SURVEY OF THE DEAD SEA AREA DURING 1942, INCLUDING THE DESCRIPTION OF A MOSQUITO FLIGHT TEST AND ITS RESULTS

BY

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The present study was undertaken at the request of the Director of Medical Services with the object of determining the source of anopheles mosquitoes found during the past few years at the residential quarters of the Palestine Potash Works at the northern end of the Dead Sea. During the course of the work the problems of the range of mosquito flight and the methods of marking mosquitoes presented themselves As will be seen later *Anopheles sergenti* were found with pollen adherent to various parts of the body The pollen was identified as *Anabasis articulata* and it is of interest to note that these naturally marked mosquitoes were caught at several places between 1 and 1¼ km. from the nearest possible source of pollen.

TOPOGRAPHY AND METEROLOGICAL CONDITIONS

The Dead Sea north end area is divided into two equal parts by the Jordan running south from the Sea of Galilee into the Dead Sea. In the west the zone is bound by the bare Judean hills to the east by the hills of Golan. This valley is desert-like, almost devoid of vegetation. The soil is of high salt content up to 17 per cent. NaCl. The Dead Sea itself being 400 metres below sea level the lowest place on the earth's surface the alley is full of depressions and dry wadies.

In summer the climate is dry and hot, the average temperature being 35 to 45 C. in the shade and up to 65 to 5 C. in the sun. A cool south wind from the Dead Sea blows daily from to 8 in the morning until 4 or 5 in the afternoon, when it changes into a north wind during the night there are mainly north-westerly winds.

TABLE I

METEOROLOGICAL CONDITIONS IN THE DEAD ST. NORTH DURING 194..

	Temperature in degrees Centigrade											
	Jan	Feb.	M.	Ap	May	June	July	Aug	Sep	Oct.	Nov	Dec.
Maximum	1 5	21 7	4 0	30·	33 6	39 5	35 6	37 4	33 9	32	·7 0	23 6
Minimum	9 6	11 0	14 5	17 1	·0 7	4 9	1 9	·6 7	22 3	2 0	17 8	12 0
Average	12	16 3	19	23 9	·7	3 1	31 7	3·0	29 1	·· 3	··· 3	1 8
Relative hum drs (percentage)	6·	6·	60	6·5	6·	43	4··	·0 3	64	55	61	6·
Rainfall in mm.	4	11·0	33 5	9 8	0	0	0	0	0	9 5	·· 0	drops

TABLE II

DIRECTION OF WINDS IN THE MONTHS OCTOBER-DECEMBER, 194.
(PERCENTAGES)

Month.	Time of the day	Direction of Winds.							
		North	North-West	North-East.	East.	South East.	South	South-West.	West.
October	Morning	63	16 7	1 4	0	4 1	4 1	0	0
	Evening	33	4 0	10 0	0	5 0	10 0	13	0
November	Morning	54 9	9	16 1	1	0	13 6	4 6	0
	Evening	3· 3	·0	4 2	1	4	8 4	1 5	4·0
December	Morning	43·0	28·0	4 0	0	1	0	0	0
	Evening	5·3	31 3	0	0	5 4	5 3	5 3	0
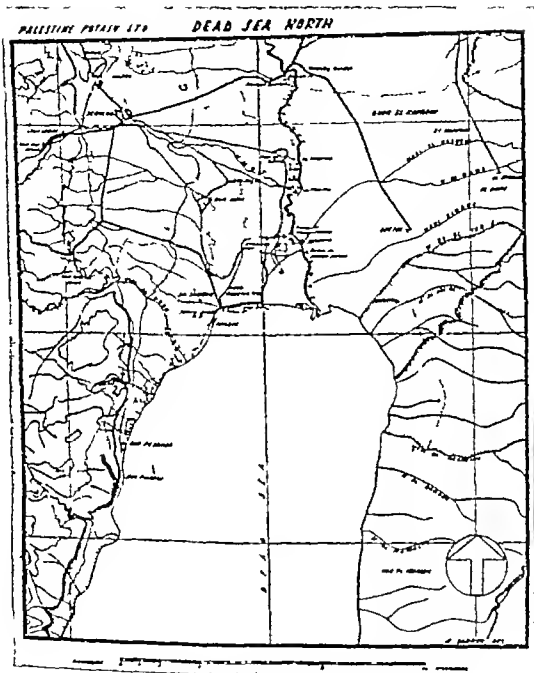
ZONE CLASSIFICATION OF THE DEAD SEA AREA.

The attached map shows the details of the Dead Sea area pertinent to this survey The area is divided into three zones —

Zone A   Kallia area comprising (1) Kallia Hotel at the southernmost point of the area   (2) the Jewish quarter   (3) the Police Post   (4) the Potash Works Compound about 1½ km north of the hotel   and (5) the Arab Workers quarter to the west of the Potash Works.

Zone B " A new settlement, Kibbutz about 3 km. north-east from the Potash Works Compound and 1 km. west of the Jordan

Zone C   Deir Hajla or Greek Convent and Hajla Farm.

SWAMPY PLACES IN THE DEAD SEA AREA.

ZONE A.

1   Several wadies shown on the map in and around Kallia area   they are dry in spring and summer and carry water in winter only

2   Wadi Umar Madib—$\frac{1}{2}$ km. north of the Arab quarter ground-water outcrops   2 5 per cent of NaCl   breeding *A multicolor*

3   The spring area marked Ein Jiyeh has been subsoil-drained from its source which is enclosed in a sealed concrete casing   There is one open trough for watering cattle   the water running through it from the spring to the subsoil area.

4   Brackish water outcrops in the form of puddles or shallow pools along the western part of the seashore found in the spring of the year   They are caused by the rise in ground water level after the rains and contain 2 5 per cent of NaCl   most of the puddles dry in summer with the lowering of the ground water level.   Breeding *A. multicolor*.

5   For purposes of this study Feshka (starting at about 4½ km. south of Kallia Hotel and stretching 3 km. further south) was also included as a breeding source   Feshka swamps form a sort of triangle   on one side there are hills at the foot of which flow about fifty springs of various sizes to the opposite side—the sea—1 km. from the hills, the third side is Wadi Kumon   The run-off of water is obstructed by elevations of gravel and coarse sand brought in by waves of the sea and puddles and seepage areas are formed along the whole length of the seashore.   The salinity is from 0 25 to 0 65 per cent NaCl   In the spring months *A multicolor* and *A sergenti* breed in the puddles along the seashore   *A sergenti* (predominating), *A multicolor* and *A superpictus* in the puddles caused by outcrops of springs and ground water obstructed in their flow to the sea by layers of coarse sand and gravel.   In the autumn and winter with the lowering of the subsoil water level, the puddles along the seashore disappear almost entirely and breeding is confined to the gravel water outcrops   the predominating type of mosquito breeding there being *A sergenti*, with a very small percentage of *A superpictus* and *A multicolor*

ZONE B

1   Wadi Kuffrin, from 4½ to 6½ km. east of Kallia area.   Breeding in spring *A multicolor* (predominating) *A superpictus* and *A sergenti*   In autumn breeding was absent or insignificant.

2   Jordan   1 km. east of the   Kibbutz   and 4 km. east of the Potash Works.   With the fall of the water level in the Dead Sea (3 to 4 m. during the past 12 years) the Jordan level also has become lowered, and in 1942 the banks were high and clear of vegetation.   This together with oscillations in the water level during the opening and closing of the dams of the Palestine Electric Company at the Jordan head   created unfavourable conditions for anopheles breeding   Prior to that, up to 11 years ago   puddles could be found

along the banks favourable for breeding of *A elutus* Along the slopes near the Jordan are outcrops of water of very high salinity, up to 25 per cent. due to seepage from the salt pans places unfavourable for anopheles breeding

3 The springs of Suama—11 km. east from Kallia area Breeding *A sergenti* and *A superpictus*

ZONE C

1 Ein Hajla and

2. Wadi Quilt from 6 to 9 km. north west of Kallia area, 3½ km. distant from the Kibbutz and 1 km. from the Greek convent. Breeding Ein Hajla—*A sergenti* , Wadi Quilt—*A superpictus* (predominating), *A sergenti* and *A multicolor* (in spring) In autumn no breeding was found (controlled and to a large extent dried up)

TABLE III

INTENSITY OF BREEDING OF THE VARIOUS ANOPHELES AT THE DEAD SEA ZONE IN THE VARIOUS MONTHS OF THE YEAR.

	Jan.	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct.	Nov	Dec
A sergenti	+	+	++	++	+	+	±	=	+	+++	+++	+
A multicolor	±	±	+	+++	+++	++	+	=	±	±	+	±
A superpictus	—	—	+	+	++	++	+	+	±	±	±	±

± Occasional larva on several dippings.   ++ 5 to 10 larvae at each dipping
+ 1 to 2 larvae at each dipping   +++ More than ten larvae at each dipping

ANOPHELES MOSQUITOES AT THE DEAD SEA AREA

Four species are found in the Dead Sea area —

A elutus Very widely spread in Palestine, but at the Dead Sea area its distribution is limited breeds in any accumulation of stagnant water appears in two seasons April to July October to December hibernates as adult. Is the most *important* malaria carrier in Palestine. Mosquito dissections in malarial areas have given 1 1 to 5 4 per cent. infectivity

A superpictus Breeds in moderately fast-running streams, on banks of rivers, in irrigation canals. Found all through the year particularly in the summer months of June to October Hibernates as adult. An important malaria vector Mosquito dissections have shown 1 1 to 8 3 per cent. infectivity But in certain cases we have found *A superpictus* in large numbers in places where no epidemics occurred, although there were both parasite carriers and a susceptible population, while if in the same place *A elutus* appeared alone or with *A. superpictus* cases of malaria were sure to follow

A sergenti   Breeds in sluggish streams and in seepage areas, in neglected irrigation canals in gravel swamps   Prevalent in Palestine from June or July on, but mostly in autumn   At the Dead Sea area it appears also during the spring months   April to June   Found during winter months both as larva and adult   It has been considered an important malaria carrier particularly during autumn   Dissections so far have given only 0 5 per cent infectivity on the other hand, the presence of *A sergenti* in numbers up to 95 per cent of the total mosquito numbers (along with *A superpictus* or *A elutus*) has given rise to marked malaria epidemics.

A multicolor   Breeds in brackish waters   While the females of the other three types do not deposit eggs in waters containing more than 0·8 per cent NaCl   *A multicolor* is found breeding in places with a salt content up to 3 5 per cent, has been found as adult in the winter   Its malaria carrying properties are not yet definitely known   It is limited in appearance as to numbers and localities.

INVESTIGATION OF MOSQUITO BREEDING SOURCE AT DEAD SEA AREA IN THE SPRING AND EARLY SUMMER, 1942.

The investigation started at the end of April, 1942, when the picture was the following   Many adult mosquitoes in the Kallia Hotel Jewish and Arab quarters, of the type *A sergenti A multicolor* with the latter predominating in the Jewish quarter and the former in the Kallia Hotel   *A superpictus* was also collected on several occasions   In the   Kibbutz, *A multicolor* (predominating) *A superpictus* and *A sergenti*   Males were found to the amount of 20 to 30 per cent in all places   In the Greek Convent and in the Hajla Farm *A sergenti*, *A superpictus* and *A multicolor*   Large numbers of culicines were also found in the Kallia area   The question was whether the adult mosquitoes in the Kallia area were from nearby local sources or whether they came from Feshka swamps, 6 to 9 Km. distant from the various places in the Kallia area.

The whole Kallia area was then combed for possible unknown local breeding sources.   No new wadies or other swampy areas heretofore unknown were found.

Variations in Water Level at the Seashore.

An interesting observation as to mosquito breeding and variations of water level was made along the western part of the seashore in the hotel area.   The shoreline at this point has a gravel bed   its extent in height and depth depends both upon the annual changes in the water level of the Dead Sea and upon the seasonal rise and fall of the ground water level.   During the past 12 years the sea has receded, and in 1942 a high shoreline was exposed, with brackish water outcrops from Kallia south all the way to the marshy

area shown on the map as Feshka springs and marsh. During a search on this shoreline in the morning just before sunrise water puddles were seen with Culex and Anopheles larvae. When the place was revisited about half-past eight in the morning the puddles had disappeared Only when we removed the gravel for about 10 to 15 cm. both the water and the larvae again became evident. The anopheles larvae proved to be *A multicolor* Salt content of the puddles was 2¼ per cent. Digging up the gravel in several other places along the seashore yielded more *Culex* larvae, and it is reasonable to suppose that on extensive search more *A multicolor* breeding could have been found It appears then that the water level along the seashore rises during the night and water appears in puddles where mosquitoes may lay their eggs after sunrise the water slowly recedes the larvae filtering through the gravel inter-spaces and develop under a layer of gravel where there is sufficient moisture the adult mosquitoes may again escape at night This phenomenon of rise and fall of water level before and after sunrise in puddles along the sea shore was noted several times during this study Although the open puddles and pools along the seashore had been oiled for a distance of about 2¼ km south of the hotel, these sub-surface breeding places had apparently not been dealt with, for they can easily be overlooked during the day A few open uncontrolled pools, along the seashore on the way to Feshka, were also found breeding *A multicolor* These as well as the sub-surface breeding places having been dealt with both by oiling and by covering with an adequate layer of earth a marked reduction in the number of culicines and of *A multicolor* resulted in the Kallia area within a short period. But *A sergenti* both females and males were still captured during May in relatively increasing numbers and the suspicion arose that the source of these *A sergenti* could not be found in the breeding places along the seashore containing 2 5 per cent. NaCl and which, so far had yielded only *A multicolor*.

We examined the area of the Greek Convent and Bir el Hajla several kilometres north of Kallia area. Although the distance to Kallia from any possible breeding source in this area could not have been less than of Feshka, it was nevertheless thought worth while looking for such sources both from a practical standpoint of local control and for the purpose of eliminating any sources capable of affecting the Kallia area. Two minor sources (pools) and one rather extensive source (Wadi Qult) were found. It is interesting to record that one of the pools covered with vegetation of the type that would usually breed *A elutus* gave 100 per cent *A sergenti* breeding Wadi Qult gave breeding of *A superpictus* 60 per cent., *A sergenti* 30 per cent., and *A multicolor* 10 per cent.

Control of these places was also started at once, so that from June onwards they could not be considered as possible contributors to the adult mosquito population in the Kallia area. For the Kibbutz " the known source Wadi Kuffrin was controlled by June with excellent results. Generally by June

the mosquito numbers everywhere dropped to negligible quantities, particularly in July and August the breeding in Feshka also became less in intensity, *A sergenti* always predominating   Further study was then postponed.

ADULT MOSQUITO BREEDING SOURCES IN AUTUMN

By the middle of September anopheles mosquitoes began to reappear in the Kallia area and their numbers gradually rose.   There were differences in the various zones in autumn as compared with spring both in the adult mosquito population and in the breeding places.   Briefly it then appeared that all the breeding places in Zones   B   and   C   (with minor exceptions) were controlled and to a large extent disappeared Accordingly by autumn the adult mosquito population dropped to negligible quantities in the houses in both zones.

In Zone A, the Kallia area proper the picture was different.   Owing to control measures and perhaps to natural changes such as greater admixture of sea water breeding places near the shore had disappeared. (A ditch left uncontrolled gave no breeding at that period.) In Feshka the puddles along the seashore had disappeared, and breeding was confined to ground water level outcrops.   *A sergenti* figured almost alone in the picture, with a very small percentage of *A ruperficius* and *A multicolor* In the houses and shelters of the various sections of Kallia area during October November and December adults of *A sergenti* only were found (occasionally a single *A ruperficius* or *A multicolor*)   These findings suggested that Feshka was the origin of the *A sergenti* at Kallia.   It is important here to record the fact that in early autumn the Arab quarter showed a relatively larger number of *A sergenti* than the Jewish quarter or Kallia Hotel.   This is, in part, explained by the fact that in the Arab quarter there were more concentrated unprotected places for mosquitoes to enter for food and shelter   the population is also larger than in the Jewish quarter or at the hotel.   In December with the general drop in mosquito numbers, the reduction was more marked in the Arab quarter and with it there was a relatively larger number of mosquitoes in the Jewish quarter   In January very few mosquitoes were found in the Jewish quarter and at the hotel, and none in the Arab quarter

Dispersion habits of male *A sergenti*

In the caves located in the hills at the western border of Feshka, males of *A sergenti* could be taken in a proportion of up to 75 per cent. of the total numbers.   In Kallia Hotel and in the Jewish quarter the males amounted to about 50 per cent. of the mosquito numbers in each place while in the Arab quarter they never exceeded 5 per cent   As stated before, no swampy area breeding *A sergenti* has been found between the Jewish and Arab quarters   The reason for this marked difference in numbers of male *A sergenti* in the

various quarters may be that while the female would tend to fly to places most suitable for feeding the males remain in the first available place of shelter In the Jewish quarter and at Kalia Hotel the mosquitoes could practically always be collected only in sheltered places as under the staircase in the lower hall or shelters outside the screened living rooms. In the Arab quarter they were taken in the tents and in hiding places very near to houses where feeding facilities existed. The presence of males in such large numbers in places so distant from the nearest breeding place indicates that the male *A. sergenti* at least in the Dead Sea area, disperse for long distances *These findings do not coincide with the accepted theory that males do not fly long distances or that their presence serves as an indication of the proximity of breeding places*

A sergenti BREEDING IN THE WINTER MONTHS OF 1943

During January there were found larvae of *A sergenti* in Feshka swamp and adults in the caves near Feshka. During our visit to the area on 20th February 1943 we easily collected eggs larvae of *A sergenti* in all stages as well as pupae in the pools of Feshka (particularly in those between the second and third springs), the temperature being 19 to 21° C. in puddles, and higher in running water We also collected in the caves of Feshka relatively large numbers of active adult *A sergenti* with fresh and partially digested blood and with developed ovaries, with eggs in various stages of development, and without any deposit of fat. At the same time there were no more puddles along the Kalia seashore, and only one or two mosquitoes could be found in the Kalia area. A feature, not observed in the spring or autumn, was the presence of camels and cattle throughout the Feshka swamps We were informed that these are usually brought down by the villagers to the swamp in the winter months probably because of lack of green in their own places or because of temperature differences

The finding of *A sergenti* in the open field.

It is worth while recording the phenomenon of finding of *A sergenti* in the open field near bushes in the early evening

This observation had first been made by one of us, S B in the autumn of 1935 He put up two huts—one 1 km. and the other 3 km. south of Kalia Hotel—on the way to Feshka, and had men sleeping in them and donkeys kept outside While *A sergenti* were found with difficulty in the huts during day time, it was easy to collect them in the huts as they came in at night in the absence of wind. On the average he collected about thirty-one *A sergenti* in the hut nearest the swamp (3 km. from Kalia Hotel) and fourteen in the other (1 km. from the hotel) During nights with strong wind no mosquitoes appeared. While sitting in the evening in the open, a short distance away from the settlement, he found that near Kalia Hotel he could collect from five to twenty in 1 hour in the absence of wind while in a field beyond and to the north of the Arab quarter or on the Jordan banks, i.e., at the extreme north end of the area possibly affected by Feshka, he could collect only from one to three, and that only rarely On 4th November 1942, in the early evening we sat in the open near a group of low bushes tamaris " about a mile south of Kalia Hotel and about 4½ km. from the middle of the Feshka swamp, and noticed that with the wind dying down or completely subsiding mosquitoes began to circle about us, attempting to bite several were collected on the skin into test tubes in or prior to the act of biting These findings were repeated on several occasions and each time *A sergenti* were collected.

c

Appended is a list of catches (all were *A sergenti*) in the open in 1942, about or shortly after sunset in the absence of wind or with a very mild breeze No mosquitoes were during a strong wind.

Date.	Time of day	Number caught.	Date.	Time of day	Number caught.
9th Oct.	After dark	12	6th Nov	6 0 p.m.	6
19th Oct.	„ „	5	7th Nov	4.30 p.m.	36
23th Oct.	„ „	1	8th Nov	5.0 p.m.	6
1st Nov	„ „	21	14th Nov	5.30 p.m.	18
4th Nov	7.30 p.m.	8			

This would indicate that mosquitoes rested in the open on their way to habitations in search of blood and since mosquitoes were collected in such places just before and shortly after darkness fell, it would further indicate that the mosquitoes could have been hiding in or rested very near the bushes during the day All these catches were in the area between Feshka and kallia. One evening—18th November 1942—we spent 1½ hours in a different direction on the north side of the Arab quarter towards Hajla and Wadi Quilt and caught only one mosquito on the donkey As previously mentioned, the swamp areas in that direction were at this time under control.

EFFECT OF WIND ON MOSQUITO DISPERSION

In the above mentioned catches in the open mosquitoes appeared not at all or in very small numbers during a strong wind, but they readily appeared when there was no wind or a very mild breeze In the table below the direction and strength of wind is given in relation to the increase or decrease in mosquito numbers in houses or other hiding places in the Dead Sea area. It is worth while recalling that the houses are in a northerly direction from Feshka swamp.

Date	Direction and Intensity of Wind.		Mosquitoes.		
	At evening of search	In the morning of search	Number	Increase	Decrease
1942					
12th Oct.	?	W.3	121		
26th	N.N W...	N.2	226	+	
8th Nov	N.N W.3	N.3	90		—
7th	S.2	N 1	200	+	
8th	S.F 0	S.4	208	+	
9th	N W 0	S.6	73		
14th	N.3	N.2	187	+	
16th	N 2	N.2	165	+	
17th „	N E.1	N.3	201	+	
18th	N W 3	N.N W.2	200	+	
20th	N.N W 1	N.E.1	137	+	
21st	S.S.E.6	S.W.5	54		+
4th	N 1	N.3	46		+

7th November and 24th November, 1942, had the same wind direction and yet the former gave a large number of mosquitoes and the latter the smallest catch in November It is seen from the table that there was an increase in mosquitoes in the houses during nights with north winds, i.e , in the direction towards and not from the swamp on the other hand there was a decrease on nights with strong south winds, i.e in the direction from the swamp to the houses. It thus appears that at the Dead Sea area —

1 The most frequent winds at night and early morning are from the north, i.e towards the swamp and away from the settlements.

2. No definite influence of wind direction on the increase or decrease in the number of mosquitoes has been noted.

3 Wind seems to have a deterrent effect on mosquito feeding activity

THE MOSQUITO FLIGHT TEST

Along with the observations on the influence of breeding in Feshka swamp on the mosquito situation in the Kallia area, it was also decided to perform the mosquito flight test from Feshka. Preparations for this test started in the spring The object was to stain for identification and then to release a large number of *A sergenti* from a point in Feshka and to look for them in the Kallia area. For this purpose it was necessary to collect mosquitoes with the least possible damage to them, preferably in the area under examination. The method of staining them had to be chosen. So far in this country only one such test had been carried out by the first two authors in 1924 who released from Birket Atta about 2,000 *A elutus* previously sprayed with a solution of methylene blue, and recovered two in Hadera, 2 5 km. away during the next 2 to 3 days. For the work at the Dead Sea methylene blue was considered unsuitable, and the use of gold dust powder was considered advisable. Accordingly, certain preliminary tests were made in the Nesher laboratory with this method of spraying and it was found that mosquitoes so sprayed continuously showed gold specks on chest and abdomen for about 2 weeks.

Following the experimental spraying of mosquitoes in cages or tubes samples were taken and all of them showed gold specks on their body particularly on chest and abdomen. Test spraying with the same result was also carried out on mosquitoes hanging in large numbers in the caves near Feshka. In the caves where the mosquitoes were sprayed in their natural environment without undergoing any physical hurt such as is liable to occur when they are plugged into tubes, the true effect of spraying could be observed and it was seen that while the floors and walls of the cave were covered with gold dust, no dead mosquitoes were seen on the floors. Gold dust was therefore adopted as a method of staining

METHOD OF COLLECTING A LARGE NUMBER OF MOSQUITOES FOR THE TEST

The method of breeding mosquitoes from larvae taken at Feshka was the first choice This was first started in the laboratory at the Dead Sea, but the larvae and adults died

out during "khamsin" (hot east winds) and in spite of all precautions ants destroyed many mosquitoes. We then transferred to the laboratory of the Department of Parasitology at the Hebrew University Jerusalem anopheles larvae and eggs, and adult mosquitoes with developed ovaries in the hope that with cooler weather conditions prevailing there a large number of mosquitoes could be bred out. But, even there the number of adults (about 4 000) required for the test could not be acquired at one time many adults dying off before others emerged and a proportion of the larvae also died. The test was then postponed till summer, since the number of mosquitoes at kallia area had dropped in June. A method for obtaining a large number of mosquitoes might have been to collect them in their hiding places near the swamps but there are no human habitations or animal sheds between Feshka and Kallia, the only hiding places being the caves along the hills to the west of Feshka. but there in spite of prepared traps, etc., collecting mosquitoes was very difficult, if not impossible, although there were thousands of them in each cave. The large percentage of males present also made it inadvisable to collect there. In one corner of the cave we counted more than 400 mosquitoes, about 5 per cent. males the rest females without blood. The mosquitoes were very active, and as sooner did we get near them than they left their resting places in masses. Attempts were made to make collections in the swamp on ourselves and on donkeys early in the morning before sunrise, but although many mosquitoes came to bite they were so active that only a limited number could be collected in the test tubes at sunrise.

Altogether 250 were then collected on donkeys and in tents amongst these there were 3 per cent males, * per cent. females with developed ovaries, 30 per cent. females with fresh blood and the rest of the females without blood. The man who stayed in the tents said that at night you could " collect mosquitoes with shovels."

It was then decided to arrange for mosquito collection in the swamp during the night. Previously a couple of tents had been set up there by the Potash Works Compound and a few men stayed in them for several nights prior to the experiment to find out the best place for attracting mosquitoes.

Mosquito collection in the swamp at night.

At last one place was selected and on the night of the 5th-6th November a group of us, fourteen men, assembled and remained there from six in the evening till eight next morning. The procedure was simple the men sat around in a circle having the legs of thighs and arms bared the mosquitoes alighting on the leg or arm was taken into a test tube alive with the aid of a torch, preferably before biting. The test tubes were collected, and the mosquitoes emptied into two cages. By morning 2,600 mosquitoes, nearly all without blood (of these eleven were *A. superpictus* one *A. multicolor* the rest *A. sergenti*) had been collected, and together with those collected in the tents of the swamp and in the houses and caves on previous days, we had a total of about 5 000 anopheles mosquitoes (*A. sergenti*) all of which developed and remained in their natural surroundings a few days before the experiment. It is important to note that none of us contracted malaria following that night's work in the swamp. This may be due to the fact that there are no human habitations between Feshka and Kallia.

Mosquito activity in the swamp at night.

Before continuing with the delineation of subsequent work it is worth while recording the following observations made during the mosquito collection in the swamp —

(1) Early in the evening the first mosquitoes to appear were culicines about half an hour later the culicines disappeared and anopheles came on the scene. Very few

culicines were seen during the night when anopheles were freely on the wing but before sunrise culicines again appeared and with them there was also a marked increase of activity among the anopheles. They literally covered the donkey

(2) During the night the anopheles mosquitoes came in waves   for a while there would be increased activity for about 30 to 45 minutes, and everybody would be busy catching mosquitoes calling for more test tubes, the men at the cages being unable to empty the tubes quickly enough to meet the demand.   Then a period of quiet would set in for about half an hour and only single mosquitoes would alight here and there until the next wave of mosquitoes would appear   These waves were independent of any change of wind   if anything the mosquitoes appeared during the period when there was no wind at all.   Generally the night was practically wind free

Spraying and release of mosquitoes

These mosquitoes were later sprayed with gold dust (a bronze preparation finely powdered) in the cages with the aid of the fine pulverizer and 1 hour later shortly before sunset, they were released through the large door of the cages at a high place in the middle of the swamp at a distance of about $5\frac{1}{4}$ km. from Kallia Hotel.   It was also found convenient to spray the mosquitoes in the caves, as they were hanging in groups on cobwebs or on stones   As a conservative estimate, nearly 10 000 mosquitoes were sprayed on that day   Before releasing the mosquitoes from the cages on the day of the test certain precautions were to be taken to prevent the masking of the natural conditions that existed in the swamp prior to the experiment   Thus, in order to avoid biting in the swamp the camp was disbanded prior to the release of mosquitoes   the tents were taken down and the men and donkeys sent out of the swamp   While waiting for the car to take us back to Kallia in the dark, we rubbed the exposed parts of our body with a   citronella   preparation which, at least for a while, prevents mosquitoes from alighting to bite

The spraying with gold dust in the caves continued for a couple of weeks following the experiment, and always the same thing was noted there on the day following the spraying   A number of stained mosquitoes and also a large proportion of new mosquitoes without gold specks was found   even if collected in places where the spraying had certainly been effective, and where samples examined following the spraying gave almost 100 per cent. results   This proved that fresh mosquitoes had entered the caves and that the mosquitoes in the caves of Feshka did not remain inactive during November but used the caves as resting places.   It also showed how great the daily changes through dispersion or change of resting places are in the mosquito population in a location supplied from a large swamp

Subsequent searches following release

Following the first stage of the experiment—the staining and releasing of mosquitoes—it was necessary to collect them in the various places at Kallia area and to examine them for the presence of gold dust.   Accordingly a staff of men were busy on the days immediately following the release and on subsequent days until 7th December 1942, collecting mosquitoes.   On the days

from 6th to 10th November 1942, and from 17th to 20th November mosquitoes were carefully examined on the spot immediately after catching : on the other days the mosquitoes were killed with ether and sent to the Nesher laboratory

The results of examination of mosquitoes are given in Table V

RECOVERY OF A GOLD STAINED MOSQUITO

In the late afternoon of 7th November at 4.30 p.m. just before dark, while sitting near a bush about 1½ km. south of Kallia Hotel, and about 4 2 km. from the place of release of stained mosquitoes, we collected thirty six mosquitoes in the act of biting Among them one *A sergenti* female showed definite specks of gold dust on the thorax and abdomen There was no mistake about it. This sprayed mosquito was thus found at a distance slightly over 4 km. from the place of release or at about a distance almost equal to that between the beginning of Feshka swamp and Kallia Hotel, on the second night after release. It also shows that the mosquito travelled a distance of 4 km. almost in one night About midnight on Saturday 7th November 1942, there was a big rainstorm which continued throughout the night, and on Sunday 8th November 1942. The catches on Sunday 8th November 1942, were probably among the highest of the season, but those of Monday 9th November were much smaller The large catch on Sunday may prove that the mosquitoes flew to the houses in the early part of Saturday night in anticipation of the storm or rather that they flew during the period of calm, resting on bushes during the stormy periods. A large number of mosquitoes must have perished during that night. The reduced catches continued through the period of 9th to 16th November when the numbers rose for a few days and then remained stationary until 7th December when examinations of mosquitoes for gold dust were discontinued. (Mosquito catches with classification as to number and kind continued through December and January 1943 but the mosquitoes practically disappeared from Kallia area towards the end of December 1942)

Except for the single mosquito mentioned above, no other gold stained mosquito was discovered. One other had some small gold like specks on the abdomen, but being too small for diagnosis this was discarded. The question may be asked why more gold stained mosquitoes did not come toward Kallia Several explanations present themselves —

(a) *The rainstorm in the night following the release of the mosquitoes could have destroyed a large number of mosquitoes which left the swamp on that or on the previous night (the night of release) resting during the day on bushes (near which the one gold stained mosquito was actually found)*

Evaluation of gold dust spraying as a method.

(b) *Although observations both in the laboratory and in the caves showed that spraying with gold dust did not kill the mosquito immediately and that it might remain alive in captivity for several days, it may also be that a mosquito liberally sprayed with gold dust cannot endure without deterioration all the trials and difficulties connected*

TABLE V

LIST OF ANOPHELES CAUGHT AT THE VARIOUS SETTLEMENTS OF KALLIA AREA ZONE A, IN CONNECTION WITH THE MOSQUITO FLIGHT TEST DURING THE PERIOD 7 11 42 TO 7 12.42.

Date.	Arab Quarter		Jewish Quarter		Kallia Hotel		Potash Works Compound.		Police Post.		Totals.	
194	M.	F	M.	F	M	F	M	F	M	F	M	F
7 11	0	1..3	1*	13	6	17	0	3	—	—	18	158
8,11	4	237	22	30	3	12	—	—	4	4	35	283
9 11	2	59	5	2	1	2	—	—	—	—	8	63
11.11	3	22	13	28	6	27	—	—	—	—	22	77
12.11	0	8	11	39	20	77	1	6	7	1	39	145
13.11	2	6	*6	23	9	19	1	13	—	—	38	61
14 11	0	4	8	12	9	8	1	13	—	—	38	61
15 11	0	10	13	18	4	3	2	8	2	5	21	37
16,11	2	85	33	33	4	3	—	—	—	—	17	42
17 11	2	120	25	71	10	16	3	10	—	—	49	155
18 11	1	60	27	65	20	49	—	—	—	—	47	229
19 11	—	—	—	—	—	—	—	—	3	9	31	137
*0 11	2	81	14	32	—	—	—	—	2	21	2	21
*1 11	0	31	3	17	2	4	0	1	0	14	18	133
22.11	0	48	13	10	—	—	—	—	1	3	3	51
23 11	*	39	4	16	2	13	—	—	1	3	16	74
*4.11	0	14	2	14	1	2	0	2	0	*	7	61
25.11	—	—	?	75	0	6	0	5	0	3	2	43
*6 11	2	31	33	*6	—	—	2	2	—	—	—	75
*7 11	—	—	4	*6	—	—	2	2	0	13	17	72
28 11	—	—	*1	19	1	1	1	4	0	9	6	31
29 11	—	—	4	21	0	3	—	—	8	7	39	29
30 11	—	—	4	21	5	6	1	6	1	7	11	40
			18	25	3	3	0	7	0	14	21	49
1 12	0	13	21	18	9	13	1	3	3	1	34	61
2.1*			8	3	1	—	0	2			29	34
3 12	0	13	1	—	1	—	0	2			29	34
4 12	2	14	*1	*4	0	16	0	13	—	—	30	66
5.1	0	17	28	.5	1	2	0	7	0	*	31	50
6.1*	0	17	11	18	1	2	—	—	0	2	13	40
7 12	5	10	17	17	—	—	0	2	0	5	22	34
			15	39	—	—	0	2	3	9	18	48
Total	30	1,069	433	785	125	235	12	94	34	157	634	2,390
Average caught daily	1	49	13	27	6	12	1	6	1	8	22	83

NOTE.—Among these mosquitoes there were 2 to 6 per cent. *A superpictus* 1 to 4 per cent. *A. multicolor* and the rest 90 to 97 per cent. *A sergenti*

with flight in open country like the Dead Sea area. If not sufficiently sprayed the powder would soon drop off. The available material, purchased in envelope packages, was not uniform in quality some envelopes contained coarser material than others, and most material was slightly moist. Just before release from the large cages the mosquitoes got the impression of less activity than usual, but this was attributed to or at least it was hoped to be due to temporary stunning It may be possible perhaps to improve upon the method and preparation of the material, but it is doubtful whether this is a foolproof method.

Pollen as a staining method will be discussed later

In the course of examination of mosquitoes caught in the various plants of the Kallia area several interesting observations were made —

1 Marked pigmentation much stronger than on any *A sergenti* observed in any other part of the country

2. The exceptionally large size of the mosquitoes in that area (*A subcolor* particularly *A sergenti*), not previously found by us in any other part of the country in the autumn.

3. The finding of pollen—*Anabasis articulata*—on the chest and abdomen of the mosquitoes (see Plate).

4 The finding of mites—Hydrachnidae—on about 3 to 5 per cent. of the mosquitoes in the Kallia region (see Plate).

POLLEN ON MOSQUITOES

Granules of yellowish green colour were found attached round the thorax of *A sergenti* mosquitoes. These granules were at first suspected of being mould or modified gold colour but subsequently they were identified at the Department of Botany of the Hebrew University as the pollen of the plant *Anabasis articulata*. Altogether about twenty five female *sergenti* mosquitoes all covered with this pollen, were collected in various places (Arab and Jewish quarters, Police Post, and in the caves at the western border of Feshka) and at distances ranging from 1 to 1½ km. from the nearest possible source of the pollen. No pollen was found on the mosquitoes sent for examination by mail it was found only when examination was carried out at Kallia on 16th to 18th November 1942, immediately after catching it may perhaps be assumed that the pollen dropped in transit.

To our knowledge there is no mention, anywhere in the literature, of mosquitoes found with pollen or that mosquitoes may take up and spread pollen.

The plant *Anabasis articulata*, from which the pollen derived, was found just outside Feshka swamp and in several other places in the field between Feshka and Potash Works. In one place, just outside Feshka swamp it was found in bloom with much pollen strewn about on the ground. In the words of the surveyor " it would be no wonder that the mosquito would pick up the pollen if touching or resting on the bush." Remembering our finding mosquitoes resting near bushes in the field it is not surprising that they picked up the pollen. One mosquito with pollen on its leg was taken in a cave during the survey it further strengthens the supposition that at least in the Dead Sea area the *A sergenti* mosquito, either by nature or by necessity has become a " rural " type of mosquito which, in quest of blood that is scarce near its source of breeding, has adapted itself to travel long distances, to rest during the day and night in caves or on bushes in an open field Certainly there would be no reason for a mosquito in easy reach of blood or shelter to rest on bushes.

This phenomenon of pollen mosquitoes is also important in that it opens
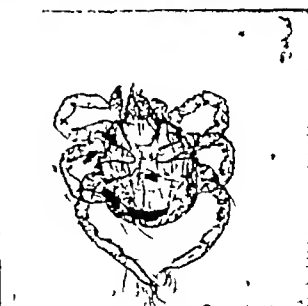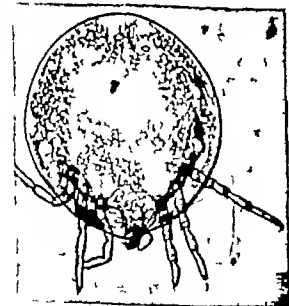
1 Mites Hydrachnidae from head of *A sergenti* × 68
2. Young larva of mite Hydrachnidae taken in the pools at Feshka Swamp × 138
3 Young larva of mite Hydrachnidae taken from a mosquito *A sergenti* (distended,
   compare as to size with the young larva taken from the Feshka Swamp) × 138
4 Pollen on leg of mosquito *A sergenti* × 740
5 Granules of pollen of *Anabasis articulata*

a new field in staining or spraying mosquitoes for a flight test and other works of identification the pollen being an organically uniform and harmless powder and one which the mosquitoes seem well able to carry especially as the botanists assure us that a coloured pollen foreign to the area to be tested can be selected. The only point to prove before use would be the length of time the powder could remain on the mosquito in actual flight under natural conditions.

MITES ON MOSQUITOES

Mites were found on about 20 to 25 per cent of all the mosquitoes taken in caves in the hills at the western border of Feshka and on about 3 to 5 per cent of mosquitoes (all *A sergenti*) taken either in the field between Feshka and Kallia or in the houses in the whole Kallia area between the period of November December 1942, when careful examination of practically all mosquitoes collected was made in connection with the mosquito flight test. Mites were found mostly on females, but also on males

Dr Shoulof, of the Hebrew University identified all the mites on the mosquitoes found in all the various places mentioned above and at various times as Hydrachnidae, i.e. fresh water mites of the Thya class This brought out an interesting question as to the source of these mites and whether they could not also serve to determine the source of the mosquitoes carrying the mites.

The first theoretical point of consideration was the degree of salinity in the various water sources in the Kallia area and their suitability for harbouring fresh water mites It will be remembered in this connection that the water in the puddles around Kallia area, when examined on several occasions was found to contain 2 5 per cent. NaCl, while the waters of Feshka contained from 0 30 per cent. NaCl (near the large spring and pools where the larvae of the mites were found) to 0·65 per cent. NaCl (the latter in the puddles near the sea) in other words even the most brackish waters of Feshka contain only one-quarter of the amount of NaCl found in the waters at Kallia seashore

The second point was that the larvae of Hydrachnidae are usually known to live in large collections of water The latter however had not been found heretofore in the actual Kallia area, but were present at Feshka or at the area near the Jordan, i.e. fish ponds, water storing reservoirs. Wadi Kuffrin and Wadi Quilt were practically dry during the period between September December 1942.

In spite of these theoretical considerations it was the opinion of Dr Shoulof that no place could be considered as a source of these mites unless the larvae of Hydrachnidae (certainly) and possibly the eggs and adults were found there since the parasites found on the mosquitoes are the young larvae of the Hydrachnidae found in the waters where the mosquitoes had developed or where they came to lay eggs.

Accordingly he examined all the known water places—small and large —from Feshka to Kallia and from there to the Jordan, i.e. for a distance north-east of Kallia almost as great as that from Feshka to Kallia It must be said here parenthetically that these collections of water have become known and mapped only after repeated previous surveys so that one could tell almost with certainty that except perhaps for a chance water collection due to a temporary leak of a tap or pipe, no other definite collections of water existed during

the period in question He found no Hydrachnidae larvae in the water places near Kallia or in the more distant neighbourhood, near the Jordan, which is as distant from Kallia as Feshka but found them in Feshka waters on three occasions, 24th December 1942, 19th January 1943 and on 20th February 1943 when he collected a great number of them in the large shallow pools of Feshka These pools apparently contain multitudes of larval mites since he found thirty five there in an area of 4 sq m. and fifteen more nearby The larvae of mites found in Feshka were similar to those found on the mosquitoes except that they were younger He also found under stones objects similar to the Hydrachnidae eggs in various stages of their development.

The conclusion here must be that the finding of mites on *A sergenti* mosquito in Feshka and in Kallia area gives a definite picture of Feshka being the source of mosquitoes which were found in Kallia area between October-December 1942. While mites on mosquitoes have been found in other places, it appears that in the Dead Sea area with its extreme climatic and geographical conditions, the presence of these mites as parasites on mosquitoes in such enormously large numbers afforded a clear method of proving long distance dispersion of *A sergenti* Since the mites were also found on males it affords further proof that the male *A sergenti* at least at the Dead Sea area, has a long range dispersion. (*Vide* note on Dispersion habits of male, page 102.)

MALARIA CASES.

Malaria cases have been occurring in Kallia area each year since the inception of the Potash Works, the number varying with the number and kind of transient labourers that may be recruited either from highly malarious areas or from those practically free from malaria. Tables VIa and VIb of malaria incidence shows that the number of primary malaria cases in a given year amounts to about 2 to 7 per cent. of the total population, the bulk of the cases occurring among the workers in the Arab quarters where there are unprotected places suitable for mosquitoes to hide in and where there is also the largest percentage of malaria carriers as compared with the other quarters in the Kallia area.

In the "kibbutz" settlement Zone B which has been in existence for the past three years, cases usually occur in the spring and summer months. As is seen from the mosquito chart (p 114), adult mosquito incidence is limited there to the spring months. This is accounted for by the fact that this settlement is affected mostly by the breeding in Wadi Kuffrin and partly by that in Wadi Quilt. Both these places, at least till the end of 1942, have been drying up either completely or for a considerable distance away from the settlement from about June or July

The cases in Zone A," Kallia area proper—usually if not always—occur in the period between October December when the mosquito incidence is higher compared with that in the spring At that time, too, as has already

TABLE VIa.

PRIMARY CASES OF MALARIA AT THE DEAD SEA NORTH
DURING 1939 to 1942.

Year	Population.	Cases.	
		Total number	Per hundred population
1939	700	48	6 9
1940	900	66	7 3
1941	1 500	21	2·0
1942	1 800	112	6 2

TABLE VIb

PRIMARY CASES OF MALARIA IN THE VARIOUS QUARTERS OF THE DEAD SEA NORTH IN 1942

…re.	Population.	Jan.	Feb.	Mar	Apr	May.	June	July	Aug	Sep	Oct.	Nov	Dec.	Total No.	Per 100 population.
1 s h er and Post A …arter	180	—	—	—	—	—	—	—	—	—	7	2		9	5
A Hotel,	800	1	—	—	1	—	2	—	1	8	64	18		93	12
A Camps	80	—	—	—	—	—	—	—	—	3	—			3	3 8
A …u t z,	820	—	—	—	—	—	1	—	—	—	3	—		4	0 7
C	120	—	—	—	—	—	—	1	—	—	—	—		1	0·0

been pointed out, *A sergents* forms about 94 to 96 per cent. of the total number of mosquitoes with about 3 to 4 per cent. of *A superpictus* and about 1 to 2 per cent. of *A multicolor* Although no mosquito dissections have been carried out, it is perhaps safe to conclude from clinical observations that *A sergents* is responsible for the bulk of the malaria cases occurring in Kallia on the Dead Sea in autumn.

DISTRIBUTION OF ANOPHELES MOSQUITOES AT THE DEAD SEA IN 1942 —
AVERAGE DAILY CATCH
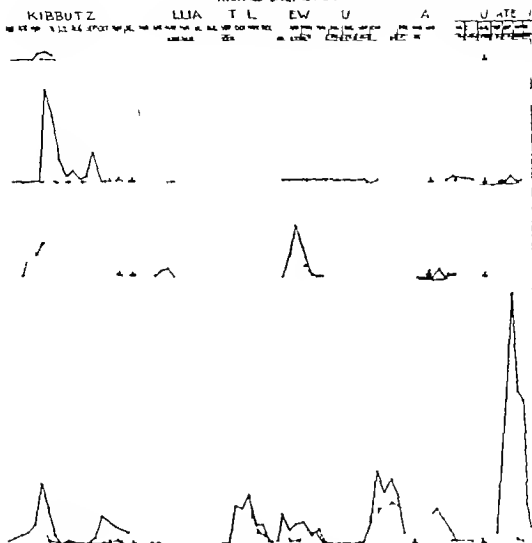
SUMMARY

The pertinent findings of the study are as follows —

1   Demonstration of breeding of *A. multicolor* and of culicines under a layer of gravel 10 to 15 cm. deep and of oscillations of water level in puddles at Kallia seashore before and after sunrise with the apparent disappearance of larvae for the day

2   Demonstration by a seasonal study of the distribution of adult mosquitoes and by elimination or control of local breeding found during the study

that Feshka, 6 to 8 km distant, is a source of *A sergenti* for Kallia area, certainly in the autumn and probably also in the spring

3   Description of a method of collecting in one night several thousand fresh *A sergenti* in the swamp   Bringing them from another source cannot be as reliable as using fresh mosquitoes from the breeding place prior to their natural flight in quest of the first meal

4   Caves are an important resting place and collecting centre for anopheles mosquitoes to an extent that has not been noticed in Palestine before.

5   Method of spraying mosquitoes with gold dust and releasing them with the subsequent recovery of one   gold stained   mosquito in the field

6   The collection of *A sergenti* in the field near bushes about sunset.

7   Observation of pollen being carried by mosquitoes—to our knowledge not recorded before.

8   No definite influence noted of wind direction on mosquito dispersion

9   The finding of fresh water mites (Hydrachnidae) from Feshka swamp in large numbers on *A sergenti* (males and females) in various localities—caves open fields and places of human habitation in the Kallia area

10   Demonstration that male *A sergenti* may travel as long a distance away from the swamps as females and that the finding of males is not always an indication of the proximity of breeding places

In conclusion, it is of value to present the factors pointing to the existence of a long distance dispersion of *A sergenti* in the Dead Sea area —

(1)   In the spring season adult *A multicolor, sergenti* and *superpictus* were collected in the Kallia area, while the breeding then found along the Kallia area seashore gave only *A multicolor* and while no other breeding source had then been discovered

Wadi Qilt could have been responsible for some adult mosquitoes in the Kallia area, but the distance from there to Kallia is not less than from Feshka. S B states that in former years when with greater possibilities for obtaining labour he kept the Kallia seashore well covered and there was no breeding *A. sergenti* was found in the settlement.   In the autumn of 1942 there were no breeding places at all along Kallia seashore and other places nearby were controlled, yet numerous *A sergenti* were collected in Kallia settlement. At the same time at Feshka *A sergenti* breeding goes on the whole year though in varying degrees at different periods of the year

(2.)   The recovery in the field of a single   gold-stained   mosquito at a distance of 4 2 km from the point of release and 1 5 km from Kallia Hotel All precautions had been taken against error in both field and laboratory

(3)   The finding of *A sergenti* in the field on and near bushes at various distances south of Kallia Hotel and in the direction of the swamp and practically no mosquitoes collected in the open about 3 km. north of the hotel

(4)   The finding of pollen on mosquitoes in the various settlements in Zone   A   may prove the migratory and rural habits of *A. sergenti* in the Dead Sea area.

DISTRIBUTION OF ANOPHELES MOSQUITOES AT THE DEAD SEA IN 1942
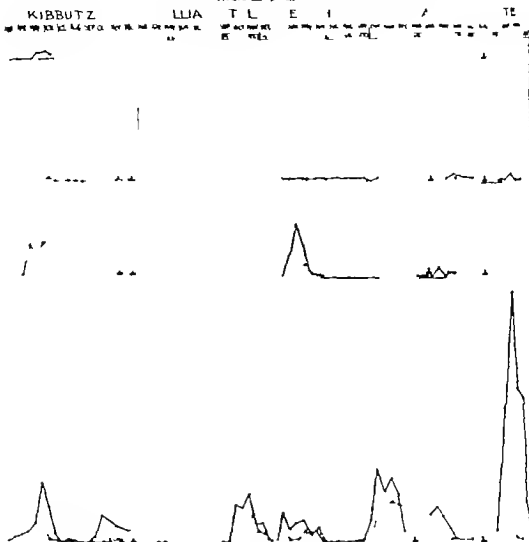
AVERAGE DAILY CATCH

KIBBUTZ   LLIA   T   L   E   I   A   TE

SUMMARY

The pertinent findings of the study are as follows —

1 Demonstration of breeding of *A. multicolor* and of culicines under a layer of gravel 10 to 15 cm. deep and of oscillations of water level in puddles at Kallia seashore before and after sunrise with the apparent disappearance of larvae for the day

2 Demonstration, by a seasonal study of the distribution of adult mosquitoes and by elimination or control of local breeding found during the study

INFANT MORTALITY IN THE BRITISH WEST INDIES

BY

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In every field of human activity it has been usual to entail definite levels of excellence, or standards of value against which progress in the direct march towards betterment might be measured from time to time—each profession occupation or calling adopting its own arbitrary standards of measurement suited to the special activities to which it is devoted

So far as Public Health work is concerned, progress can only be effected measured provided services have the support of the intricate structure of vital statistical machinery capable of producing dependable rates or indices which numerically reflect the various aspects of life and death characterizing communities existence. It follows that the reliability and value of such rates will depend upon the exactness and range of the raw material made available for analysis and interpretation, plus a willing determination to make full use of vital rates in that effort when these requirements are fulfilled the available rates are of primary importance in the framing of future administrative and medical policies in the interests of health betterment which may reasonably be expected to follow with promise of success

Among the many quantitative assessments regularly used by Public Health authorities, *the infant mortality rate** is regarded as an exceptionally sensitive measure for the purpose of determining the efficiency of health administration since a high infant death rate is held to reflect the combined effects of

* That is the ratio of the total deaths of children under 1 year per 1,0
registered during the same year

conditions and the evil influences of a variety of external circumstances gener inimical to health and life * These truths are common knowledge, and p in many parts of the world records of infant deaths are lamentably incomple and unreliable with the result that authorities are denied the valuable inform tion and help which could be made available if only the regular maintenan of dependable records were insisted upon throughout the services. Lacki complete and dependable records, public health officers faced with recurren high or steadily increasing infant mortality rates, may endeavour to soot anxiety by seeking to attribute such unfortunate experiences to the operatio of some inscrutable Act of God and refuse to recognize the fact that unneces waste of infant lives may largely be the logical consequence of their own defin

Despite the great strides in social and medical progress in various par of the British Colonial Empire in recent years, it cannot be denied that exce sively high infant mortality rates continue as an annually recurrent experien in too many of our overseas possessions. Speaking generally such rates ran from about 60 to 600 per 1 000 births many of the published rates mu however be accepted with considerable reserve for records are frequent incomplete and unreliable or may refer only to selected areas where registrat is enforced and may thus represent but a very small proportion of the who population within the boundaries of the territory

For example in some of our possessions in British Tropical Africa registr tion of vital facts is not attempted except for Europeans in other territori where registration is applied in selected areas only these requirements aff from 2 to about 10 per cent. of the total population of the territories who such practices have been introduced. To quote an actual example, it may observed that the population of the Colony of the Gambia numbers appro mately 200 000 yet records of births and deaths, etc., are available only for t capital city of Bathurst with a population of about 14 000 where the inf mortality rate which was reported to be 184 1 per 1 000 births in 1938 h risen to 243 3 per 1 000 in 1941 † Of the rates obtaining in the Protector where 92 per cent. of the total population of the Gambia are to be foun nothing is known or can be known until reliable registration systems are est lished there and made to function efficiently

On the present occasion it is proposed to focus attention upon inf mortality experience in a group of territories where civil registration system have been established for a long time and where requirements are constan in use and uniformly applied to all sections of the population inhabiting the territories. The territories in question comprise a reasonably compact ge graphical group in the Western Hemisphere where were laid the foundatio and where are to be found some of the oldest units of our Colonial Empir

* As tests of sanitary conditions the death-rates of infants under 1 year and children under 5 years, are more important than the rates in any other groups of ag Vital Statistics, Farr Memorial Volume p. 114 London 1885.
† Cf. Annual Medical Report Colony of the Gambia for the year 194

namely the British West Indies. For all practical purposes the distinctive features—population climate, vegetation, etc.—characterizing life in British West Indian Islands may be regarded as similar despite inevitable variation to be met with between island and island, and even at times between different areas of individual islands.

All the available data—some of them of doubtful accuracy—relating to thirteen principal territories in the British West Indies were assembled for the 5-year period 1934–38 and average infant mortality rates calculated for each, these are arranged in descending order of magnitude and presented in the table below with other statistical indices which experience has shown usually to vary either directly or inversely with the rate of infant mortality

Colony	Infant Mortality Rate (a)	Birth-rate (b)	Coloured Births per cent. of All Births	Illegitimate per cent. of Total Births	Births. Coloured	Of Total Population per cent. Coloured
Barbados	221 6	29·4	No data	59·0	No data	93 3
St. Kitts (c) ...	163·9	38·9	"	No data	"	96 8
Bahamas "	146 5	31 4	"	"	"	80·0
British Guiana ...	138·6	32 1	93·4	49·7	"	98 7
British Honduras	135 1	35·0	74·6	43 9	"	97(?)
Jamaica	129·6	31 9	No data	71·6	"	98 3
Antigua "	124·6	36 4	98 7	74·0	"	85·6
St. Vincent	118 1	39 3	No data	70 3	"	95·5
Trinidad (d)	107 7	31 8	41 8 (East Indians)	89·4	86 5 (East Indians)	99 3
Grenada	105·4	31 3	No data	63 4	No data	98·6
St. Lucia	102 5	31 7	"	65 1	"	?
Dominica	100·8	30·9	"	57·0	"	93 5
Montserrat	96 7	35·2	99 5	65 2	"	99·0

(a) Per 1 000 births Reports do not always indicate whether *live births* only or whether records include an unstated number of stillbirths.

(b) Per 1 000 population.

(c) Indicating the Presidency comprising the islands of St. Christopher with Nevis and Anguilla.

(d) Classification of births, etc. confined to (1) East Indians, (2) other than East Indians.

It has not been considered necessary to calculate the coefficients of association between the infant mortality rate and other indices[*] for a glance at the above table shows no statistical relationship in those cases where complete or approximately complete data are available for examination. For example,

[*] Cf A. NEWSHOLME (1923) *Vital Statistics* 3rd Edn. Chap. X, pp 112–22. M. GREENWOOD and J W BROWN (1912) *Some Factors influencing the Rate of Infant Mortality J Hyg.*, 11, pp 5–45

D

Indies, yet its average infant mortality rate of 118 1 per 1 000 births is the highest in the group   moreover infant deaths in St. Vincent claim an average annual toll of approximately 30 per cent. of the total deaths at all ages, a much more unfavourable experience than those of St. Lucia or Grenada.

Trinidad which lies about 10 degrees north of the Equator is the second largest and the most southerly of all the British West Indian Islands. The climate is hot and damp and malaria is prevalent. The average infant mortality rate for the 5 years under review was 107 7 per 1,000 births, the infant deaths during the period claiming about 20 per cent. of the total deaths recorded each year

It remains to discuss whether further examination of the available data can throw light upon some of the causes of infant mortality and the wide variations of experience noted in these islands. Further investigation supplies the following information.

LEGITIMACY AND INFANT MORTALITY

It is a well-established fact that the deaths of illegitimate infants are proportionately about twice as numerous as those born in wedlock. Unfortunately the majority of unmarried expectant mothers lack the care and attention enjoyed by married women during the pregnancy period and after birth takes place illegitimate babies are often denied the attention and nourishment necessary for their survival. In the preceding pages attention has been drawn to excessively high infant mortality rates, and the large proportions which infant deaths bear to the deaths at all ages in some British West Indian territories the latter feature is in fact largely responsible for the high general death rates experienced in those territories.

It may be noted from the table of comparative data presented on page 119 that *in the British West Indies illegitimate births invariably form the larger proportion of the total births recorded each year* Here, then, may lie one cause of unduly high infant mortality in these islands, though it also may be noted that there is no constant relation between infant mortality rates and the percentages of illegitimate births. For instance, British Honduras and British Guiana have the lowest illegitimate percentages, yet they occupy fifth and fourth places respectively in the West Indian infant mortality experience on the other hand, in Montserrat, where the proportion of illegitimate births is 43 per cent. higher than in British Honduras, the infant mortality rate is 28 per cent. lower, but in Barbados the proportion of illegitimate births is 34 per cent. higher and the infant mortality rate 64 per cent. higher than the corresponding rates in British Honduras. These variations must not be interpreted to mean that illegitimacy is *not* an important cause of infant mortality in these islands*—from the available data all that can be said is that illegitimacy *is* a factor but that its

*Cf. *Local Government Report* (New Series), 1912. Part III. pp. 46–48. London H.M. Stationery Office.

relative importance when compared with other influences cannot be determined with accuracy in the present study

Births are classified by legitimacy (or the percentage proportions of illegitimate births are stated) for eleven of the territories under discussion Of the total births recorded for these eleven territories during the period 1934–38 illegitimate births accounted for 66 per cent. In *Antigua* during the 5 year period 74 per cent. of the births were illegitimate in *Jamaica* 71·6 per cent. in *St Vincent* 70 8 per cent. and thereafter in descending magnitude to *Barbados* with 59 per cent. *Dominica* with 57 per cent. *British Guiana* 49 7 per cent and *British Honduras* with 43·9 per cent. *Why* should the high proportion of illegitimate births be a regularly recurrent phenomenon in British West Indian colonies ? The general view suggests that the high average is mainly due to the fact that among the less educated classes a man and his unmarried mate both work to earn wages for the common use of the family they are raising since the usual custom is for a woman to cease work outside the home on being married If this view is substantially correct then it would appear that the average wage of the married West Indian labourer is insufficient to provide the ordinary necessities of life, and consequently widespread poverty must characterize the lives of the majority of the people in these islands West European experience has shown that birth rates, illegitimate rates, and infant mortality rates, provide sensitive indices of poverty for where statistics of births and deaths are classified by social status it has been noted that birth and infant mortality rates are highest among the poor and lowest among the well to-do [*] In this connection it is not without interest to observe that such rates are consistently high in West Indian territories. Poverty is a highly complex phenomenon resulting from the inter play of a variety of elements and influences and these in action have the effect of producing not only excessive infant mortality but equally they may be responsible for the creation and maintenance of the unpleasantly high ratios of illegitimacy which are an outstanding and regularly recurrent feature of the vital statistics of British possessions in the Caribbean Sea.

In *British Guiana* where over 80 per cent. of all East Indian births are illegitimate, this high percentage is said to be due to the fact that there exists no legislation for the recognition of Hindu marriages with the Protector of Immigrants under the provisions of the Immigration Ordinance On the other hand, the recognition of Muslim marriages is provided for under the Muslim Marriage and Divorce Registration Ordinance of 1935 provided such marriages are effected by and before a duly appointed Muslim Marriage Officer and registered under the terms of the Ordinance (According to the results of the Census of 1931 Hindus were about five times as numerous as Muslims) These differences of practice are difficult to understand surely the determination of

[*] Cf. GREENWOOD and BROWN loc. cit. p 23 *Local Government Report* loc. cit. pp 54–56 GREENWOOD and BROWN loc cit. pp 20 31–33 NEWSHOLME, loc. cit. pp 292–294

legitimacy for registration purposes should be governed solely by the real rules of the particular population group to which newly born children belong

Unfortunately with two exceptions only it is not possible to assess numerically the effects of illegitimacy upon infant mortality in British West Indian colonies, for though births are classified with distinction of legitimacy in similar classification appears to have been undertaken for infant deaths in the majority of published reports relating to the territories under review. The necessary data are available for *Barbados* and *Jamaica* and for the period 1934–38 it was found that in Jamaica 78 per cent. of all infant deaths recorded during the 5 years were those of illegitimate babies, while in Barbados the corresponding proportion amounted to 71 per cent. These figures are depressingly suggestive of the destructive possibilities of the illegitimacy factor in the problem of infant mortality in British possessions in the Caribbean Sea.

RACIAL COMPOSITION AND INFANT MORTALITY

The term Race is here used in a very general sense for mixed marriages during the past three centuries in the West Indies have tended to modify and obliterate racial distinctions in many of the existing human stocks inhabiting the islands. Perhaps a more general classification of the population as " White " and " Coloured " might be the wiser plan to adopt for present purposes but whatever technical expedient is applied it is clear that obscure racial factors supply influences of outstanding importance in this problem of infant mortality

It is to be remembered that among a total population of some three millions in the British West Indies, barely 4 per cent. are Europeans and Whites, the remainder being mainly of African or mixed descent, with in some territories a considerable sprinkling of immigrant and locally born East Indians. The Bahamas has a larger proportion of resident Whites (20 per cent.) than any other British West Indian colony * Thereafter the proportions of white to black or coloured peoples range from about 1 to 7 or 8 per cent. while among the coloured elements further variations may be noted as, for example, the numbers of East Indians in British Guiana and Trinidad and so on.

At this point it may be appropriate to observe that in countries where the population comprises both white and coloured elements infant mortality is almost invariably higher among the coloured races For example, in the United States of America the rates in 1936 for Whites and Negroes were 52·4 and 88·1 per 1 000 live births respectively while for individual States the rates ranged from 41·2 to 63·9 for Whites and Other Races in Connecticut to 112·9 and 303·8 for the corresponding racial groups in New Mexico.†

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* The small island of Barbuda (which with Antigua and Redonda forms one of the Presidencies of the Leeward Islands) is perhaps the only place in the West Indies where the majority of the inhabitants are Whites.

† *Birth, Stillbirth and Infant Mortality Statistics*, 1936. U.S. Dept. of Commerce, Bureau of the Census, Washington, 1938

we turn to the Far East we find that in Batavia (Dutch East Indies) during the period 1935–37[*] infant deaths among Natives accounted for 36 per cent. of the total deaths recorded, for Chinese 33 per cent., and for Europeans only 9 per cent.[†] while in Sumatra (also Dutch East Indies) similar experiences were recorded.[‡]   In the Federated Malay States of British Malaya in 1939 the infant mortality rates were   Europeans (and Eurasians) 51 7, Malays 147 Chinese 115·4, and Indians 121·6 per 1 000 respectively §   But when attempting to unravel the intricacies of the infant mortality problem in British West Indian territories, the investigator is faced with a disheartening task owing to the lack of essential facts and the absence of any uniform system of classifying basic data , in many official reports no attempt is made to tabulate natality and mortality data according to race or colour

Bearing in mind the vital statistical inadequacies which feature so many of these Reports, it may be noted that in *Barbados* about 75 per cent. of the births are those of coloured infants, and in *British Guiana Antigua* and *Montserrat* about 98 per cent.   in *Trinidad* and *British Guiana* of the total births recorded, the contributions by East Indians alone amount to some 58 per cent. and 48 per cent. respectively   It would appear that infant mortality rates among the coloured elements of the inhabitants of West Indian territories are invariably in excess of those relating to the infants of the white inhabitants but until steps are taken to improve existing vital statistical machinery and methods of reporting   precise assessments cannot be made   as matters stand the majority of official reports fail to supply the necessary data for the determination of specific infant mortality rates.   So far as *Barbados* and *British Guiana* are concerned it was found that during the period 1934–38 mortality rates of coloured infants were on the average about 50 per cent. less favourable than the corresponding rates for white babies   In *British Guiana* in 1938 the infant mortality rate for Europeans was 69 per 1 000 births as compared with a corresponding rate of 179 for East Indians, 171 for Blacks, 130 for Mixed Races, and 109 for Aborigines.   Other colonies do not supply information in this detail   the general practice is to quote an infant mortality rate for a colony as a whole without distinction of colour or race

Unduly high infant mortality rates among the coloured inhabitants of these islands are almost certainly due, in part at least to the fact that the majority of these people are lamentably ignorant of or they ignore, the most elementary laws of hygiene and sanitation.

[*] J D DE HAAS (1939)   *Mortality According to Age-Groups in Batavia, etc. Ind J Pediatrics* Vol. 6 October

[†] No compulsory registration of native births in Batavia therefore not possible to calculate I.M.R. correctly per 1 000 births.

[‡] M. STRAUB (1928). *Kindersterfte ter Oostkust van Sumatra*, Amsterdam.

§ *Report of the Registrar-General Federated Malay States 1936*   Government Printer Kuala Lumpur 1940

DISEASE AND INFANT MORTALITY

When this aspect of the problem is approached the investigator once again finds he is condemned to traverse the dreary plains of meagre information. Individual authorities in the British West Indies seem to cling with tribal jealousy to their own parochial views of what items of knowledge concerning the phenomena of human life and death are necessary for publication in official reports with the result that scientific enquiries are frustrated, for nomenclature of causes of death are almost as varied as the contents of a witch's cauldron making comparability of mortality experience between colony and colony virtually impossible. In three colonies only of those under review are causes of infant deaths reasonably classified, in two others only two titles of cause of death are adopted, in another two no attempt was made to classify infant death with respect to cause before 1937. In two more lists are presented intermittently, in one colony five titles forming the group " Diseases of Early Infancy " is an early revision of the *Detailed International List of Causes of Death* are used, and in the remaining colonies no attempt whatsoever is made to supply information relating to the causes of infant deaths.

On the basis of the very slender resources available it was found that *congenital disability* appeared to be responsible for the majority of infant deaths in these islands a term so vague as thus conveys no useful information. In *Trinidad* 45 per cent. of all infant deaths appear under this title for the 1934-38 period, followed by *Grenada* and *St Vincent* two of the islands of the Windward Group with 39 8 and 38·9 per cent. respectively then *Jamaica* 33·9 *British Guiana* 27 2 and *St Lucia* 25·9 per cent. Next comes *Barbados* with 17 2 per cent. and the remainder in order of descending magnitude to the *Bahamas* where only 0·3 of all infant deaths are ascribed to this cause.

Premature birth was reported to have claimed the deaths of 15·4 per cent. of the total infant deaths in *British Guiana* during the 5-year period under review Next in order come *Trinidad* and *Grenada* each with 10·9 per cent., *Antigua* 10 8, *Barbados* 8 per cent., *Montserrat* 7 5 per cent., *St Vincent* and the *Bahamas* each 5 2 per cent. and *Jamaica* with the lowest recorded percentage of 2 7 per cent. for this cause.

As has already been observed, in three colonies only are more than two causes of infant deaths classified. In *Barbados* 30 per cent. of all infant deaths were ascribed to *diarrhoea and enteritis* in *St Lucia* 19 7 per cent. of the deaths appear under this title, and in *British Guiana* 10·3 per cent. It is curious to note that while over 15 per cent. of all infant deaths are classified as due to *syphilis* in *Barbados* and *St Lucia* the percentage in *British Guiana* is only 1 3. *Respiratory diseases* in *Barbados British Guiana* and *St Lucia* were responsible for 13·6 12, and 5 7 per cent. respectively of all infant deaths during the period 1934-38.

Malaria as a cause of infant deaths is listed only in reports from *British Guiana* and *St Lucia*. In *British Guiana* 18·9 per cent. of all infant deaths were

assigned to ' fever (probably malaria),' and in *St Lucia* malaria accounted for 5 3 per cent. of the total infant deaths   It is particularly unfortunate to find so little information in the reports under discussion regarding the effects of malaria on the lives of infants in British West Indian territories where malaria is endemic , many of these infant deaths occur within a week or 10 days of birth and may largely be due to untreated malaria in expectant mothers who are prematurely delivered of their babies while suffering attacks of malarial fever

The above results make it sufficiently clear that classifications of causes of infant deaths in the majority of the islands under review, are so inadequate as to make them comparatively useless for public health purposes. It has been seen that where only two causes of death are tabulated *congenital debility* and *premature birth* appear to claim an undue proportion of infant lives but this result may be the dangerously misleading consequence of faulty human book-keeping   other causes such as *diarrhoea and enteritis syphilis respiratory affections* or *malaria* may inadvertently have been overlooked through being in cluded in these two widely embracing terms and their relative significance as destroyers of life unrealized The unwisdom of accepting published figures at their face value thus becomes apparent. Then again under many administrations in various parts of the tropical world causes of death may be certified by both medical and non medical officers and the resulting records aggregated and classified without distinction of source   The reliability of conclusions reached after the study of such treatment of disparate returns must always be suspect, and in any case the data made available by the exercise of such practices cannot pretend to supply any dependable measure of the diseases menacing life within the boundaries of a territory

So far as the units comprising the British West Indies are concerned it therefore becomes necessary to discover what proportion of the total deaths registered in the various territories are certified as to cause by qualified medical practitioners and, where medical certification is not complete, whether steps are taken to classify separately the returns of medical and non-medical certifiers

CERTIFICATION OF CAUSE OF DEATH

Of the official reports examined relating to the thirteen territories included in this survey eight gave no assessment of the proportion of deaths medically certified as to cause   five gave the desired information regularly

In *Barbados* and *Trinidad* over 98 per cent. in *British Guiana* over 60 per cent. in *British Honduras* about 50 per cent. and in *Jamaica* over 45 per cent. of all deaths registered were medically certified during the period 1934–38

So then it would appear that in these islands a considerable proportion of the total deaths registered have the cause of death certified by non medical persons—in some cases the certification of cause may even have been recorded by the certifier viewing the body *after death has taken place* The end results of these practices are reflected in the pages of published official reports where

the facts recorded by medically qualified and non-medical certifiers are classed together without differentiation—with one exception to be referred to later as to source of origin. In these circumstances the recorded facts are of little value in furthering public health progress, for they cannot pretend to supply dependable information of cause of death   as the assembled facts lack an essential attribute of uniformity   no inter island comparability of mortality experience with respect to cause of death is possible

But these are not the only defects characterizing the data under review. Different rules of selection of a cause of death when more than one morbid condition is recorded on the certificate, and different methods of classification and tabular arrangement of the raw material, all combine to complicate and further the problems of comparability.

For in these islands a variety of methods of classification of causes of death (at all ages) is encountered. In four territories during the period 1934-4 causes were classified by a nomenclature of 200 titles and eighteen main disease groups according to the *Detailed International List of Causes of Death*, Fifth Revision. In five others only sixty five specially selected titles were used, and in one case among these, deaths *non medically certified* were further classified by a list of thirty two disease titles and uncertified deaths by a list of ninety-seven titles alphabetically such arranged, ranging from " Asthma " to " Worms ", and containing such indeterminate terms as " Pain in the Side,   Cough,"   "Bad Bowels,   etc. with seventeen references to   Fever " in association with various diseases. In another territory 202 titles were used in another fifty two, and another all causes were represented in eighteen main disease groups which had the effect of completely masking specific causes of death. These divergences of practice combine to baffle all attempts to study the causes of infant mortality or to compare differences or mortality experience between these islands.

Concluding Observations

The present survey has succeeded in doing nothing more than indicate that though many of the recorded facts may be of doubtful accuracy sufficient evidence has been assembled to suggest that infant mortality rates in some of the British West Indian possessions are unpardonably high, and that these rates show widely uneven differences between the various units under review   it has failed in its quest to point a finger to the specific causes responsible for the majority of infant deaths in these islands   it has failed to discover what are the infant mortality rates of the poorer classes and the corresponding rates for those sections of the population more comfortably situated from the environmental and economic points of view so that the effects of such influences cannot be assessed.

The only general conclusion to be reached is that high infant mortality where they occur are the inevitable accompaniment of insanitary conditions poverty ignorance the bad living conditions of the people and a host of other

attendant ills. While these matters are primarily the concern of the various public health authorities they are additionally everybody's business, for no population can evade their communal responsibilities when conditions inimical to health and life are allowed to persist from year to year

While official vital statistical reports fail to present the facts necessary for the detailed study of this and other problems of the highest public health importance, scrutiny of *Annual Medical Reports* provides additional and complementary information which serves to throw light on various aspects of the infant mortality problem. It is clear that in many of the territories under review, sanitary conditions are little less than offensively primitive. The large towns—though not by any means all of them—may be provided either wholly or in part with water borne sewage systems, but elsewhere earth, trench pail or barrel privies, and other equally objectionable methods of sewage disposal, are the common practice. Even where attempts have been made to install water-carriage systems of disposal to septic tanks, these attempts have often proved depressingly unsuccessful abysmal ignorance and placid indifference so frequently lead the people to dispose of old clothes, etc. in the closet pans with consequent choking of drains. In the face of such disheartening experiences it is scarcely surprising to find health authorities deciding that extensions of septic tank systems could not be encouraged.

Faulty methods of disposal of excreta are undoubtedly and mainly responsible, especially in rural areas for the high incidence of *enteric dysentery* and *other bowel diseases* and for widespread *helminthic infections* In some rural areas over 70 per cent. of the people were infected with hookworm the type mainly affected belong to the poorer classes who rarely wear shoes and indeed, medical officers frequently report that few of these patients ever wear boots or shoes except on holidays. For the rest, infections with helminths other than hookworm, appear in many places to be the rule rather than the exception.

So far as *water supplies* are concerned, while many of the large towns have piped supplies and modern purification plants, it can be said that in some areas even piped supplies are not considered entirely safe, while in others, supplies which are precarious and uncertain are received from unsafe sources In country districts catch water systems tanks, ponds, rivers stored unprotected barrels, etc., are the sources of supply for the large majority of the people. The torrential rains which characterize certain seasons of the year in these islands have the effect of washing soil and other debris into collecting receptacles and ponds from which domestic supplies are drawn, with the result that *dysentery* and *enteric* are a not uncommon consequence. Polluted supplies are largely responsible for the prevalence of *gastro-intestinal* ailments in these islands, and in this connection it may be noted that in most of the territories under review the large majority of the deaths ascribed to such diseases are those of infants.

In spite of the constant attention devoted to the problem in recent years, *housing conditions* of the poorer and coloured classes still remain in many areas

deplorably primitive insanitary and overcrowded. It is true that most dwellings continue to be erected as funds become available, but their number are drastically inadequate, and even when provided, are too frequently misused by their tenants. Here, the authorities have to contend with a persistent ignorance of the elementary rules of health, for there exists a characteristic dislike of ventilation and fresh air not only will night air be excluded from superstitious fear of "jumbies" or ghosts, but this tendency extends to the closing of the vents by means of which fresh air at any time may enter and ventilate dwellings with adverse effects upon the health of the inhabitants. Unsavoury slums comprising unsightly shacks in ruinous condition and grossly overcrowded are a common feature of areas in the neighbourhood of larger towns and in rural districts. In practically all territories housing and slum clearance schemes have engaged the attention of the responsible authorities for many years, yet the problems of re-housing are not easy of solution, for until some means is found for erecting suitable houses in sufficient numbers and at rents the labouring classes can reasonably be expected to pay large-scale demolitions of insanitary dwellings and slum clearance could only result in rendering more families homeless, or in still further increasing the existing overcrowded conditions in many areas.

The question of housing is inseparably associated with *conditions of labour* in these islands where the several communities are mainly dependent for their subsistence upon agricultural pursuits. Even in years of exceptional prosperity supplies of labour invariably exceed demands, and in any case it is only during the cropping seasons that a fair amount of labour is usually employed. Between the seasons large numbers of persons are therefore unemployed, and it may be noted that during the periods of economic depression which have so frequently characterised conditions in these islands between the two great wars unemployment became virtually general. Where, in such circumstances as these, employment is discontinuous, life for the average individual becomes a precarious business, widespread poverty is inevitable, and it becomes altogether impossible for the people to afford the economic rents of houses really fit for human habitation, even if such houses were available in sufficient numbers which at present they are not. Meanwhile these unfortunate people continue the unequal struggle for bare existence as best they can. The large sums of money distributed with depressing regularity year by year in some of these islands for poor relief reflect to some extent the magnitude of the local problems of poverty confronting the authorities in one territory the cost of poor relief administration each year exceeds the total cost of all medical and sanitary services of the colony!

In view of what has been said in the preceding paragraph it would follow that many of these people would find themselves unable to purchase regular and adequate *food supplies* and would in consequence be compelled to lead a miserable existence on starvation diets and unsuitable foods. The poorer

classes and unemployed are, as a general rule unable to afford the purchase of milk or meat, and their diets which are largely composed of starchy foods lacking the essential protective elements are deficient in proteins and fats, while apart from the poverty factor ignorance is mainly responsible for the loss of food values through improper preparation and cooking of the raw materials. So far as infant lives are concerned, it may be noted that as milk is so rarely purchased for babies other and unsuitable foods provide the underlying cause of many infant deaths. It is a common practice of many West Indian mothers of the poorer and labouring classes to feed their babies even during the first weeks of life, on locally grown and prepared arrowroot, flour pastes, mashed bananas etc. with unfortunate results for the children gastro-enteritis and digestive ailments which are exceedingly common and exact a considerable toll of infant lives, are undoubtedly due to these improper feeding practices.

Insanitary conditions, poverty and ignorance are only some of the factors of first importance to be considered in great detail if this urgent problem of infant mortality is to be properly understood and solved *syphilis* is probably responsible for a large percentage of the infant deaths recorded in these islands (as it may prove to be for practically all stillbirths and premature births) for whatever the *certified* cause of death may be, investigation may prove that syphilis is often an underlying cause. The *fly nuisance* in some West Indian territories is an affliction to be experienced if its importance as a danger to health and life is to be adequately appreciated fly-borne infections are so often a main cause of dysentery diarrhoea and enteritis and it is not without interest to note that in some of these islands the regular recurrence of summer diarrhoea in infants is locally referred to as fly diarrhoea. '

But for the investigation of the innumerable influences responsible for high infant mortality official reports as at present compiled are of little help to earnest workers anxious to assist in trying to unravel some of the difficult problems of health and causes of death peculiar to our overseas possessions. The important matters under discussion cannot be assigned to the sole care and responsibility of the personnel of Maternity and Infant Welfare services, for though such services are established with varying degrees of completeness and efficiency in the majority of the territories under review they are insufficient in numbers and inadequately equipped and staffed to meet the present existing demands for advice and help Moreover, some of these services are administered by voluntary organizations and as such may be wholly or partly free from any sort of control or supervision by the official Medical Department of a colony in such circumstances, while unfailingly recognizing the value of their work and the debt of gratitude due to the public-spirited benevolence of these voluntary workers, it is submitted divided control neither serves the best interests of a community at large, nor can non-official voluntary services be as efficiently organized, equipped, staffed and operated as they should be as an integral part of a central Department of Medical Services.

Until administration of all Maternity and Child Welfare services is organized upon uniform lines and becomes the sole charge of the central medical authority and until uniformity of practice in the assembly analysis and classification of vital facts is insisted upon and all civil registration services is placed under the direction and control of local Medical Departments, Public Health authorities in these islands will continue to be denied the invaluable aid of the medico-statistical instrument. Meanwhile out-dated official machinery will continue to labour mightily and succeed only in producing vital statistical reports which are largely so much printed waste.

TYPHUS RESEARCH IN EGYPT, PALESTINE, IRAQ AND IRAN

BY

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Below is a description of work done in the Middle East between December, 1941 and March, 1944. It is divided into five parts.

  I   Serological tests, with special reference to the inter-relationship between the Weil-Felix test and the Rickettsial Agglutination Reaction (R.A.R.)

  2.   Isolation of strains of rickettsiae by animal inoculation.

  3   Enzootic murine typhus in Egypt and Palestine.

  4   Discussion.

  5   Summary and Conclusions

SEROLOGICAL INVESTIGATIONS

These have been performed on sera obtained from typical severe cases of typhus fever in British and Dominion troops serving in Egypt, Palestine,

Syria Iraq and Iran and also from civilians in the same areas. (See Table I)
A preliminary account has already been published (VAN ROOYEN and B[?]-
CROFT 1943) describing the results in seventy three cases observed dur[?]
the winter typhus epidemic of 1942, during which year 21,879 civilian ca[?]
of typhus were notified in Egypt. The present report describes the final re[?]
of research which has continued throughout the 1943 epidemic when 40[?]

TABLE I

INCIDENCE OF TYPHUS IN THE CIVILIAN POPULATION OF EGYPT.

Year	Jan.	Feb.	Mar	Apr	May	June	July	Aug.	Sep.	Oct.	Nov	Dec.	Tor[?] case[?]
1940	183	501	1 183	667	778	436	133	99	30	30	25	70	4,[?]
1941	428	833	2,174	1,680	1 721	1,032	323	164	33	31	238	453	8,[?]
1942	1 199	2,318	4 237	4,797	4 748	2,414	749	323	1 9	126	323	569	21,[?]
1943	2 077	3 232	4 744	9 733	9 045	5,311	3,717	1 088	349	244	176	344	40,[?]

INCIDENCE OF TYPHUS IN THE CIVILIAN POPULATION OF PALESTINE.

Year	Jan.	Feb.	Mar	May	May	June	July	Aug	Sept.	Oct.	Nov	Dec.	Tor[?] case[?]
1940	9	5	7	12	14	7	29	39	44	29	33	16	5[?]
1941	13	8	12	9	19	20	29	46	49	49	40	30	3[?]
1942	6	10	7	6	17	15	30	31	31	79	4	20	2[?]
1943	19	12	18	33*	72*	63*	37	19	33	23	23	22	30[?]

* During April, May and June at one centre in Palestine there was a small outbreak of epidem[?]
typhus imported from Egypt.

cases occurred. A further 200 sera have been studied and the data [?]
summarized in Tables II and III.

Technique of the Weil Felix and Rickettsial Agglutination Reactions.

The three antigens employed consisted of standard R.A.M.C. Prot[?]
OX19 suspensions, and two highly concentrated and purified suspension[?]
rickettsiae, one of which was prepared from an epidemic strain origin[?]
isolated by Major J C. SNYDER of the U.S. Army Medical Corps, during [?]

Spanish Civil War, from a victim at the Commendores prison Madrid, and the other from a murine case studied by the late Professor ZINSSER in Mexico Both epidemic and murine strains had subsequently been propagated in the yolksac of the egg embryo by Dr JAMES CRAIGIE of Toronto University Canada. Through the courtesy of Dr CRAIGIE and Prof R. D. DEFRIES of the University School of Hygiene, liberal quantities of well-washed suspensions of concentrated rickettsial have been gifted to the Middle East Army, and with them the rickettsial agglutination reaction has been tried and the results compared with the Weil-Felix test under practical field conditions Mouse lung rickettsial antigen prepared by Major JANET NIVEN, R.A.M.C. has also been tried out and found satisfactory

Three parallel series of dilutions of patient s serum of 0 4 c.c. bulk, varying from 1/100 to 1/6400 were made up in 0 43 per cent. saline containing 1/20 Sorensen s $(KH_2PO_4/Na_2H PO_4)$ sodium potassium phosphate buffer of pH 7 2. To the first an equal (0 4 c.c.) volume of *Proteus* OX19 antigen diluted 1/15 in buffered saline was added. To the second and third a similar volume of epidemic and murine rickettsial antigen was added. The density of antigen employed approximated to that of a Brown s opacity tube, standard I Thus each patient s serum was tested in triplicate for evidence of agglutinins against *Proteus* OX19 epidemic and murine rickettsiae. Subsequently all mixtures were incubated for 4 hours at 42 C placed in the ice chest overnight and the results read next morning

In the case of the Weil-Felix test, a positive result is easily visible. With the rickettsial agglutination reaction it is sometimes necessary to hold each tube before a pointolite lamp and rotate briskly between finger and thumb to see the end point of agglutination. If difficulty is experienced in reading the result, the supernatant fluid should be aspirated, a film made from the deposit, stained by Macchiavello's method and examined under the low-power lens of the microscope for signs of clumping Agglutinated rickettsiae appear as masses of regular size (in comparison with clumps of agglutinated *Proteus* OX19) and appear as a fine granular flocculum which settles to the foot of the tube, leaving clear supernatant fluid above. Dreyers tubes with tapering ends are eminently suitable for the purpose.

Results of earlier Weil-Felix and rickettsial agglutination tests by VAN ROOTEN and BEARCROFT (1943) showed that, of a total of fifty carefully studied military and twenty three civilian cases of typhus investigated, the majority agglutinated *Proteus* OX19, and a few clumped OX2 although no evidence of tick typhus could be elicited. With severe Egyptian epidemic typhus showing high-titre OX19 reaction, the homologous epidemic strain of rickettsiae were agglutinated to equally high titre. With sera derived from mild Syrian and Palestinian murine typhus high-titre OX19 results were also returned and the murine rickettsiae were frequently clumped in a greater serum dilution than the OX19 reading No cases of mite-borne typhus showing OXK response

TABLE II—Continued

SUMMARY OF RESULTS OBTAINED IN EGYPT PALESTINE AND SYRIA

Case No.	Locality	Date of Onset.	Date of Test.	OX19	E.R.	A.L.R.	Diagnosis
77	Aleppo	16.6.43	14 7 43	800	800	<40	Epidemic
78	Geneifa	1 7 43	19.7 43	1,600	3,200	200	Epidemic
79	Beirut	3 7 43	15 7 43	1,600	1,600	200	Murine
80	Alexandria	27 6 43	17.7 43	3,200	3,200	800	Epidemic
81	Kantara	11 7 43	21.7 43	1,600	400	400	Murine
82	Syria	6 7 43	21.7 43	4,000	1,600	400	Epidemic
83	Syria	2 7 43	21.7 43	4,000	1,000	<40	Epidemic
84	Quassassin	12.7 43	26.7 43	200	400	400	Murine
85	Quassassin	10 7 43	26.7 43	800	200	1,600	Murine
86	Quassassin	12 7 43	26 7 43	1,600	400	3,200	Murine
87	Quassassin	12 7 43	26.7 43	100	100	800	Murine
88	Quassassin	17 7 43	26.7 43	200	<40	800	Murine
89	Quassassin	16 7 43	29.7 43	200	1,600	400	Epidemic
90	Quassassin	16 7 43	29.7 43	200	200	1,600	Murine
91	Suez		26.7 43	800	200	1,600	Murine
92	Suez	20.7 43	1 8 43	400	800	3,200	Murine
93	Kantara		3 8 43	400	400	1,600	Murine
94	Quassassin	27.7 43	2 8 43	800	1,600	400	Epidemic
95	Quassassin	20.7 43	2 8 43	400	100	400	Murine
96	Quassassin	20 7 43	3 8 43	3,200	800	1,600	Murine
97	Quassassin	27.7 43	3 8 43	800	200	400	Murine
98	Quassassin		3 8 43	800	800	800	Murine
99	Quassassin	0 9.43	8 8 43	400	400	200	Murine
100	Quassassin	27 6 43	8 8 43	200	200	1,600	Murine
101	Geneifa	18 7 43	8 8 43	400	200	1,600	Murine
102	Suez		9 8 43	200	100	1,600	Murine
103	Geneifa	27 7 43	9 8 43	1,200	400	<40	Epidemic
104	Quassassin	31 7 43	11 8 43	200	200	800	Murine
105	Ismailia	2 8 43	12 8 43	800	400	100	Epidemic
106	Quassassin	1 8 43	11.8 43	200	1,600	3,200	Epidemic
107	Geneifa	31 7 43	13 8 43	200	800	3,200	Murine
108	Geneifa	28.6 43	14 8 43	800	200	1,600	Murine
109	Suez	31 7 43	14 8 43	800	100	800	Murine
110	Suez	16.7 43	14.8 43	200	400	1,600	Murine
111	Haffasua, Syria		16 8 43	800	800	200	Epidemic
113	Quassassin	1 8 43	16 8 43	400	800	100	Epidemic
114	Suez	7 8 43	19.8.43	1,200	800	400	Epidemic
115	Suez	7 8 43	20.8.43	800	200	200	Epidemic
116	Quassassin	17 7 43	9 8 43	400	<40	400	Murine
117	Suez	5 8 43	21.8.43	3,200	200	200	Murine
118	Cairo	6 8 43	29.8 43	200	200	1,600	Murine
119	Suez	15 8 43	26.8.43	800	400	1,200	Murine

TABLE III

A SUMMARY OF CIVILIAN CASES OCCURRING IN IRAQ AND IRAN DURING FEBRUARY TO APRIL, 1944

Locality	Total cases treated.	Epidemic Typhus.	Murine Typhus.
Amman	3	3	0
Baghdad	24	22	2
Iraq—Persa Frontier	4	4	0
Teheran	50	46	4
For all above areas	81	75	6

derived from the same epidemic area, were tested under identical conditions.

Specificity—Both the Weil-Felix and rickettsial agglutination reaction (R.A.R.) tests are highly specific for typhus fever

VARIETIES OF TYPHUS FEVER IN MIDDLE EAST AS REVEALED BY AGGLUTINATION TESTS

Proteus OX19 is the principal proteus strain agglutinated in Libya, Cyrenaica Egypt, Palestine, Syria, Transjordan Iraq and Iran. Excepting Palestine and Syria, where endemic typhus is of the mild, murine type, in which murine rickettsiae are agglutinated to high titre, epidemic louse borne typhus seems to be the prevalent disease in the rest of the countries named, and this has been proved in two ways first, by the large number of cases which agglutinate suspensions of epidemic rickettsiae, secondly by the isolation of twenty-eight epidemic strains of infection in guineapigs in Egypt and thirty-one strains by one of us (J H BOWIE) in Bagdad, Mosul and Teheran. It is of interest to recall that the strain of rickettsiae embodied in the epidemic suspension supplied by Dr CRAIGIE was originally isolated by Major SNYDER in Madrid Theoretically, a higher degree of specificity may be expected of antigen prepared from indigenous rickettsiae, but this does not necessarily follow

Time factor in the Development of Agglutinins in Typhus

According to FELIX (1941), in 75 per cent. of cases of typhus the Weil-Felix test becomes positive in the 3rd to 6th day of illness One s own experience has proved different. The careful researches of CROFTON and DICK (1944) on the Weil Felix test in Egypt should also be consulted at this point. In British and Allied troops under the vigilant eye of R.M.O s, base hospital specialist medical officers and pathologists of the R.A.M C an accurate case history and date of onset of the rash (usually on the 5th day of illness) is generally obtained. From review of a 100 or more clinical notes, there is no doubt that on the 4th or 5th day the Weil-Felix result is invariably below the normal titre of 1/100 and should be regarded as negative in laboratory routine work. Also the rickettsial agglutination test is negative at this stage when using foreign strains of epidemic rickettsial antigen. It remains to be seen whether locally isolated rickettsiae would be more sensitive On the 6th and 7th days a low titre OX19 and similar positive rickettsial agglutination occasionally may be observed to a serial dilution of 1/200 There is great difficulty in differentiation between epidemic and murine type agglutination at such a stage of the disease when the Weil-Felix test reading is of a low order On the 8th and 9th day the two reactions are usually positive to a titre of 1/400 From the 10th to 14th day both reactions become strongly positive and many vary from 1/800 to 1/6400 The practical point to be emphasized is that, if a suspected case of typhus has been ill for longer than 10 days and the Weil-Felix titre is below 1/500 the clinical diagnosis should be reconsidered forthwith. From the 14th to 21st day very high titre OX19 and rickettsial agglutination is invariably present in severe epidemic typhus. So-called typhus fever without rash and with a negative Weil-Felix test throughout, has not yet been detected in the Middle East.

Identification of Type

The rickettsial agglutination reaction is superior to the Weil-Felix test in that it permits of the differentiation between epidemic and murine varieties of infection. On the other hand the antigen used is exceedingly costly to manufacture. Furthermore owing to the small size of the rickettsiae, flocculation is difficult to see unless the serum employed is one which has a high OX19 titre so that differential agglutination is marked. For the latter reason the rickettsial agglutination test gives optimum results 5 to 10 days after the rash has appeared—namely the 10th to 15th day of illness.

Animal Inoculation Experiments.

Isolation of Rickettsiae

Twenty-eight different strains of epidemic typhus were isolated by guineapig inoculation from human cases and lice on patients in Egypt. In Persia and Iraq one of us (J H. Bowie) recovered thirty-one strains from human blood and lice. The Egyptian strain of rickettsiae causes a mild febrile illness in the guineapig with no gross naked eye or histopathological lesions save congestion of the peritoneum, enlargement of the spleen and high temperature of 105 to 106° F about the 9th to 10th day after inoculation with infected blood. Films of peritoneal exudate show abundant mesothelial cells, lymphocytes and occasional polymorphs, but intracellular rickettsiae are very hard to find. Scrotal swelling has occasionally been observed but never orchitis matting or adhesion of the tunica vaginalis. Epidemic strains recovered by Major Bowie at Mosul, Sulaimaniya in Kurdistan and Teheran in the Elburz mountain district of Persia were more pathogenic to the guineapig, causing pin-point haemorrhages into the peritoneum, accompanied (as Bowie has demonstrated) by infiltration of the myocardium and typical aggregates of cells resembling classical typhus nodules described in human brain. The possible difference is being probed further and may well be due to greater infectivity or exalted virulence, in primary isolation. Craigie has succeeded in infecting egg embryos with Persian strains of rickettsiae and the causal agent thus identified. One particular strain (11A1) isolated in Teheran by Bowie was passaged in Egyptian bred guineapigs eighteen times. Petechial peritoneal haemorrhages were observed during the first five transfers but gradually then diminished, and at present, although inoculated animals develop high fever 105° F on the 8th to 9th day haemorrhages have vanished. It is possible that repeated passage in guineapigs is accompanied by attenuation in virulence.

The above findings have been compared with standard strains of murine typhus isolated in guineapigs by Dr Aschner of the Hebrew University Jerusalem, and Professor Dennis of the American University Beirut, and there is no doubt that the presence of orchitis and scrotal swelling observed in Palestinian and Syrian strains provides a sharp contrast with the absence of such lesions in Egyptian, Iraq and Iran types.

Attempts were made to recover strains of *R mooseri* from six cases of clinically typical mild murine typhus in Egypt. Only one was positive, and oedema and scrotal swelling were produced in guineapigs, but unfortunately it failed to survive secondary passage. Evidence supporting the existence of human murine cases in the Suez Canal area thus rests on R.A R. results alone. It is very interesting to recall that a similar experience was encountered by Plotz *et al* (1943) working at Kingston, Jamaica in the British West Indies. Here, Plotz and his co-workers described sixty eight cases of murine typhus on the basis of specific complement-fixation reactions, since efforts to isolate the agent from a few patients were not successful. Such evidence indicates that whilst *R. prowazeki* can readily be isolated from humans *R mooseri* tends to be elusive and is hard to recover. The reason for difficulty in transmission of *R mooseri* infection from man to guineapig is hard to understand. It is conceivable that owing to the short and mild nature of the malady many cases either evade recognition or are diagnosed at the time when patient's blood has become non-infective.

The Serological Response of Guineapigs

Animals infected with blood from six Egyptian and six Iranian patients (showing positive epidemic rickettsial agglutination) were tested serologically for development of agglutinins against homologous antigen. Such animals on the 16th day after inoculation likewise showed agglutination of epidemic rickettsiae from 1/500 to 1/1000 with lower titre murine reaction. A positive OX19 reaction was never observed in guineapigs—a fact already well established.

Value of Guineapig Inoculation.

Since introduction of the rickettsial agglutination test the value of guineapig inoculation for ascertaining whether a patient or group of cases occurring in any area of infection is suffering from epidemic or murine typhus, has diminished. During the course of the present investigation several hundred guineapigs were injected, but this was done to demonstrate the interrelation between the pathological effect of guineapig inoculation and the specific human serological response towards suspensions of rickettsiae. In military medicine there is one set of circumstances which demands prompt animal injection, namely when a single severe case suddenly arises in an otherwise healthy unit. If such an occasion did present itself and the patient were likely to die early in the disease before the Weil Felix and rickettsial agglutination response had time enough to become positive, there is an added risk that naked eye or microscopic lesions may not be visible at autopsy. Thus the results of guineapig inoculation may offer the only chance of establishing a retrospective diagnosis of epidemic typhus, and so provide justification for the institution of elaborate precautionary hygiene measures. Four such instances occurred.

Attempts were made guineapig and thereby attempt to isolate a strain of clinically typical mild In Palestine, KLIGLER and COMAROFF (1936) recovered oedema and scrotal from wild rats captured at a farm colony In Turkey it failed to survive id in Tunis, SPARROW (1937 1937a) did likewise, MOOSER, human human case, (1931) also demonstrated the presence of *R. mooseri* is very interesting, is captured at a prison in Mexico City *et al* (1943) works to this time-honoured practice is that it is too slow costly, PLOTZ and his impracticable to examine more than a few rats at a time under the basis of sp of study with the result that positive findings are insufficient agent from a at percentage of rats at large are infected.

whilst *R. pr* nd approach which has been extensively adopted by public health to be elusive working at seaports of the Mediterranean littoral and elsewhere, of *R. mooseri* use of the Weil Felix test. The latter possesses the advantage conceivable a larger section of the rat population to be sampled—but suffers either evad awback that the normal laboratory-bred white rat may contain natural has become for *Proteus* complicating the issue and introducing an element of y into the results.

ly, LECCISOTTI (1938) found that forty-seven out of ninety-three sera from rats captured in Taranto harbour agglutinated OX19 in a dilution 1/100 also ZWIEZI (1938) reported that 16 per cent. of rats from an area reacted positively

the studies now in progress direct rickettsial agglutination tests for of murine infection have been employed and by the use of egg culture tial suspensions prepared by CRAIGIE, of Toronto and by mouse lung well nded by Major JANET NIVEN R.A.M.C. of the Army Emergency Everleigh, sera from 270 Egyptian and 1 044 Palestinian to a strain of *R. mooseri* has been isolated by guineapig Since introduction o single *R. norvegicus* (captured in Port Said docks) pig inoculation for ascertain egative Weil Felix but a positive rickettsial in any area of infection is sure s CURBELO *et al* (1941) have reported a diminished. During the course o

guineapigs were injected but this w esults   The interpretation and signi-
between the pathological effect of guinea, of interest to the academician and
serological response towards suspensions e purpose of the present discussion
there is one set of circumstances which concerned, the Weil-Felix test is
namely when a single severe case sudde test seems to be more specific
unit. If such an occasion did present itself f positive returns. (3) Of 270
early in the disease before the Weil Felix and past or present typhus infection.
had time enough to become positive, there ending a more exhaustive survey
microscopic lesions may not be visible at aut of enzootic rat typhus in Quassas-
pig inoculation may offer the only chance of es id, has constituted the source of
of epidemic typhus and so provide justificati among the military encampments
precautionary hygiene measures   Four such It had been our intention to carry

TABLE IV

RESULTS WITH RAT SERA

Locality	Number Tested.	Number positive.			Date of Tests.
		Proteus OX19	Murine	Epidemic	
	Controls	Normal, white laboratory bred.			
From Cairo	56	18	0	0	19.L.43
From Cairo	1	0	0	0	1.12.43
From P.H. Laboratory Jerusalem	10	0	0	0	1.1.43
Totals	71	20	0	0	
		Wild Rats			
Haifa Port and Town	100	Not done	12	0	24.11.43
Tel-Aviv Town	54		9	0	1.1.43
Haifa	64		11	0	1.12.43
Kallia on Dead Sea	34		0	0	
Haifa Town	105	3	16	2	30.11.43
Haifa Port	22	1	5	0	30.11.43
Tel-Aviv Town	110	9	11	0	1.12.43
Tel-Aviv Suburbs	30	5	7	1	19.12.43
Haifa	50	11	12	4	7.1.43
Tel-Aviv	83	0	8	0	27.1.43
Jaffa and Tel-Aviv	186	15	26	0	8.1.44
Haifa	132	14	10	0	10.__44
Haifa	46	7	7	1	3.3.44
Totals	1 016	63	125	14	
Camp Quassasin	4	0	0	0	22.8.43
Geneifa	18	0	9	0	7.8.43
Fayid	2	0	1	0	25.8.43
Geneifa	2	0	0	0	4.8.43
Quassasin	10	0	1	0	17.43
Beni Yousef	16	0	4	1	9.10.43
Docks of Port Said	76	3	24	0	6.L.43
Cairo	120	9	1	0	10.1.43
Giza Village	24	0	0	0	
Totals	270	1	42	1	

Technique.

In view of the large number of tests done direct slide spot tests have been used and found to be satisfactory

A positive result signifies the presence of specific agglutinins in serum dilution of 1 : 25 and over

out a similar survey of the rats at Suez where many human cases had occurred, but owing to the appearances of bubonic plague in the town we reluctantly refrained from doing so at the time. Palestine has long been regarded as the home of typical mild murine typhus and it has come as no surprise to find that of 1 044 agglutination tests done on rat sera, 135 positive reactions were found. The areas from which the rats hailed comprised those where many human cases had been contracted and included Haifa, town and docks, Tel Aviv—Jaffa and suburbs.

To sum up from the above results it may be stated that a technique has been devised whereby it is possible to obtain an approximate estimate of the prevalent rat typhus carrier rate of an area where human cases are notified. To what extent war conditions have aggravated and intensified the circumstances favourable to the proliferation of rats in ports and the overcrowded homes of native labour is hard to surmise. So many factors have to be reckoned and it is well nigh impossible to be dogmatic, but in all probability it seems most likely that murine typhus has existed among rats and man in ports of the Eastern Mediterranean basin for many a long day. The short and mild nature of the malady is probably one reason why its presence has been overlooked. One suggestion for the future of interest to the clinician and hygienist would be to keep a sharper look-out for possible cases of murine typhus with particular reference to all cases of unexplained P.U.O. contracted within the precincts of the Mediterranean seaports. If it were possible to perform routine Weil Felix and R.A.R. tests in all cases whether suspicious of typhus or not, the outcome of such an enquiry might prove illuminating.

Human Murine Typhus

In Egypt the disease is most prevalent in the Suez Canal area during summer months of July, August and September. Likewise the same is applicable to Palestine and Syria. It is well to remember that each human case represents an accidental case contracted as the result of a flea bite and thus the crop of cases which develop during the late summer season may conceivably be correlated with the life history and bionomics of *Xenopsylla cheopis*. In theory the remedy to the situation is simple and calls for the wholesale destruction of rats but it must doubtless be equally plain to experienced officers of Field Hygiene Sections and others who are in contact with grim reality in Eastern countries that the slaughter of these pests presents an insurmountable task.

The Study of Rat Typhus in Relation to Anti typhus Vaccination.

Two different typhus egg culture vaccines are in use at the present time —CRAIGIE's Toronto preparation, which is a polyvalent product consisting of both epidemic and murine antigens, and the American manufactured articles,

which are monovalent and possess a single epidemic component like the Weigl louse vaccine. Confronted with these two alternatives, the prospective user is faced with having to make a difficult choice. The simplest line to adopt would be to employ the appropriate vaccine in each area. Thus for example, in Central Europe, where epidemic disease constitutes the prevalent variety, the corresponding monovalent epidemic suspension can be expected to provide a certain amount of immunity. In Egypt, CRAIGIE's composite vaccine is perhaps the best in view of the existence of both the epidemic and the murine disease and the present work on rats and human sera strongly supports this belief. Unfortunately the position is not as simple as the above would believe one to suppose. It has long been held by ZINSSER and others that murine typhus antigen conferred some immunity against the epidemic disease. Likewise it has been shown that although, in the normal course of events, strains of epidemic typhus were non-orchitic in the guinea-pig (whereas the murine type produces such lesions), if lice were infected with murine virus the latter was liable to undergo permanent alteration in characteristics so as to simulate the non-orchitic epidemic variety. Recent unconfirmed work in China by LIU and ZIA (1941) has suggested that the body louse could function as a carrier of murine rickettsiae and so cause epidemics of louse borne murine typhus. It is quite possible that the same state of affairs may prevail in parts of Egypt where both epidemic and murine typhus coexist. But so far murine rickettsiae have never been isolated from lice and meanwhile, since bubonic plague has occurred in the same area as that of murine typhus, it is likely that the flea is responsible for both diseases. Thus until such a time as more is known about the subject, in the writer's opinion it may be prudent to adhere to the older view and employ bivalent vaccines, particularly in Egypt, until the monovalent one is proved to be superior.

DISCUSSION

EPIDEMIC AND ENDEMIC TYPHUS FEVER IN THE MIDDLE EAST

Severe louse-borne *R. prowazeki* infection has invariably been regarded as the *epidemic* type and the mild murine flea-transmitted (*R. mooseri*) disease as the *endemic* variety (see PHILIP 1943). The work of BRILL (see GORDON 1940) was the first to disclose that epidemic louse borne typhus could exist in endemic form among certain sections of the American public who had probably contracted the disease during their early childhood in Europe. Subsequently MAXCY (1926) revealed that in the south eastern United States, two varieties of endemic typhus existed, the one murine typhus and the other Rocky Mountain spotted fever.

As far as Libya, Egypt (cities of Cairo Tanta, Alexandria) Transjordan, Iraq and Iran are concerned, the maximum case incidence is in March, April and May—when the state of lousiness among the population probably attains

its peak. Over a period of two years' study rickettsial agglutination and guineapig inoculation tests have proved that the bulk of the infections were of the epidemic louse-borne variety In September and October when the typhus rate fell to its lowest, epidemic type infections still persisted. Thus severe epidemic louse borne typhus constituted the indigenous 'endemic form for typhus in Libya, Egypt, Transjordan, Iraq and Iran Cases are usually severe, but mild ones are very common and should not be regarded as murine typhus for clinical reasons alone. (See Table III p 138)

In the Suez Canal zone, the epidemiological picture is a complex one because this area is inhabited by a perpetually shifting human population and cannot be treated as a semi-closed community Conflicting factors to be reckoned with are the influx of native labour from Upper Egypt (alleged to be the home of murine typhus according to local doctors) and the big cities, seeking employment at the ports, the mass movements of British, Allied and Dominion troops to and from the area, and the unknown behaviour of the rat, louse and flea population. Serological results indicate that both epidemic and murine typhus do exist, the former being more prevalent in the winter and the latter in the summer The transition from epidemic in winter to murine in summer is illustrated in Table II (p 137)

In Palestine and Syria the position has been different August, September and October seemed to show the maximum case incidence with negligible mortality Both rickettsial agglutination and guineapig inoculation tests proved that the relatively few cases which developed were of the murine type, and in consequence the indigenous Palestinian disease may be regarded as of the mild murine endemic type which apparently does not attain epidemic proportions. Thus each case of murine typhus represents an accidental infection as MACKENZIE (1941) has pointed out. (Table I, page 134)

It is unfortunate that the two terms epidemic and endemic have been so indiscriminately applied to typhus in the literature. It will contribute towards the better description of typhus if the terms epidemic and endemic were discarded and instead the disease labelled according to the insect vector responsible e g louse flea, tick- and mite borne typhus respectively The introduction of the rickettsial agglutination brings such a classification within the bounds of reality Moreover the test provides a convenient method whereby the geographical distribution, seasonal variation and type incidence of typhus in different parts of the world can be surveyed.

Mode of Infection in British Troops

Popular belief sponsored by unrevised views expressed in medical textbooks, has been responsible for perpetuating the idea that only the lousy are exposed to infection.

In the case of native labourers who develop typhus, every man may be

lousy and likewise the degree of infestation among the community from which the patients come may be equally bad.

With British officers and other ranks not a single instance of case to case infection has been reported and many proved infections have developed without the slightest history of the individual being lousy at any time. Thus from accurate clinical notes on 200 British patients, in only six were a few lice found and these men hailed from base units employing large numbers of civilians.

The question arises, how do such persons free from lice, contract typhus? One answer is that they have been unfortunate enough to be bitten by a single infected louse which has escaped notice—by no means an unlikely event. There is, however, another explanation. Dried louse excreta has long been under suspicion and shown to harbour and actually preserve the virus for several days. The following single experiment was instructive. The garments of a native victim were removed, placed on a tray about a tablespoonful of lice collected, and the remaining powdery red desiccated excreta brushed off the seams with a feather. Both the lice and dried faeces were emulsified separately in saline, guineapigs were injected and two strains of epidemic virus were isolated with ease. In certain units, personnel working in close contact as natives in factories, workshops, wharves, canteens and those visiting crowded cities must have repeated exposure to the infection by the inhalation of infected louse faecal dust. It is likely that a small proportion of such persons may acquire typhus in this fashion. Concrete evidence is hard to produce, but from what one has seen, it is a possibility considering the numbers exposed to infection and afforded opportunities. Needless to say after a patient has been deloused, shaved and placed in a clean hospital bed, all danger of infection ceases.

Anti-typhus Vaccination in Two Aspects

The present investigations have a direct bearing on the rational use of typhus vaccines. First, by the R.A.R. a systematic study has been made of 273 cases disseminated over a vast territory during a period of two years. Valuable data have been accumulated respecting the epidemiology, season variations and type distribution of the disease in the lands mentioned. Secondly, in the Middle East, it has been proved that the epidemic and murine antigenic components of CRAIGIE's vaccine (as used by the M.E.F.) react brilliantly in vitro agglutination tests against human sera collected from the field in which vaccine (made from the same source) is employed. Time alone will tell if anti-typhus vaccination is to be of real benefit, meanwhile mass inoculations should not be practised in any country without first surveying the terrain and subsequently verifying the authenticity of the antigens to be utilised. Finally in Egypt, Iraq and Iran active immunization against typhus should be performed in November so as to give protection during the ensuing epidemic period December to June, when the risk of infection is greatest.

SUMMARY AND CONCLUSIONS

1 Sera from 273 cases of typhus fever occurring in Egypt Palestine Iraq and Iran have been examined for evidence of agglutinins for *Proteus* OX19 *R. prowazeki* and *R. mooseri*

2 The specificity of the reaction has been checked by the performance of 292 control tests on 130 normal and 162 sera from diseases other than typhus

3 Continuous investigations have extended over a period of 27 months and included two successive winter typhus epidemics in Egypt, Iraq and Iran

4 Rickettsial agglutination tests reveal that epidemic typhus recurs annually from January to April in the countries mentioned.

5 With the approach of hot weather in May severe epidemic typhus subsides and is replaced by the mild murine variety of infection

6 A special study has been made of the Suez Canal zone. It is suggested that the high incidence of human murine cases is attributable to the presence of enzootic typhus among the rat population of the area.

7 The rickettsial agglutination reaction (R.A.R.) provides a convenient method whereby the wild rat typhus carrier rate of any locality may be surveyed

8. Of 1 044 sera, collected from wild rats trapped in Palestine, 135 showed evidence of agglutinins for *R. mooseri*

9 A strain of murine typhus was isolated from the brain of a rat (*R nor egicus*) captured in Port Said docks

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Such a test takes months to complete, and is of little value for the routine examination of chemical compounds. Soong and Anderson (1941) have confirmed the findings of Wang *et al* with neostibosan and solustibosan, and have set forth principles which should be fulfilled by leishmanicidal tests. Kurt and Schmidt (1938 1941) have published the results of several tests on neostibosan and solustibosan, and have shown that in European hamsters the interval between doses has an effect upon the results of a test. Liver puncture before treatment and postmortem examination of spleen and liver smears were used as criteria of infection and cure. Adler and Tchernomoretz (1940 1941 1942) have used the spleen as an index of infection in Syrian hamsters and these workers were the first to attempt quantitative tests of any degree of accuracy. Fragments of spleen were removed under anaesthesia before and after drug treatment, and the number of parasites per 100 splenic nuclei of all types counted in stained smears. In recent work by Fulton (1944), the number of parasites per 100 fields of a spleen smear has been used as an index of infection but no accuracy is claimed for the results.

Experiments in this laboratory have confirmed the difficulty of interpretation of liver puncture material which must have led Adler and Tchernomoretz to use spleen biopsy as the method of choice. Also it has been shown that with several different strains of leishmania, single subcutaneous doses of effective drugs exert a considerable action upon the degree of infection in the spleen, and a rapid therapeutic test has been devised for leishmanicidal substances. Before an accurate quantitative test can be formulated, however, it is essential to know what variation is to be expected in the index of infection used. The ratio of parasites to spleen-cell nuclei used by Adler and Tchernomoretz shows most promise as an accurate means of assessing infection, the number of nuclei serving as a scale against which to measure the number of parasites. These workers, however, have reported no investigations upon the reliability of their method.

The work presented below is an attempt to assess the importance of various sources of error which could affect the use of parasite spleen-cell nuclei counts. Later reports will deal with the technique and results of a quantitative therapeutic test in which the errors of the results can be calculated, and also with the response of various strains of leishmania to drug treatment.

The Spleen as an Index of Infection in the Syrian Hamster

A difference in parasite count in the spleen before and after drug treatment, upon which a quantitative test might be based, could be caused by a number of factors other than a real reduction in intensity of infection due to drug action. All these must be considered before a reliable potency test can be devised.

1 Counting an inadequate number of parasites and nuclei would give a result with too large a variance.

2. Uneven distribution of parasites in the spleen the microscopical appearance of sections suggests that this is important.

3 Sporadic changes of infection

4 The effect of drug treatment may be greater in some areas of the spleen than in others.

METHODS.

Animals The hamsters used in these investigations were the Syrian species, *Cricetus auratus* and were all bred in England from the descendants of the animals brought from Palestine by Dr E. HINDLE. They were fed upon a war time diet of bread, oats and vegetables with cod liver oil, yeast extract and mineral supplements.

Leishmania. The strain of leishmania (Strain A) used was isolated in culture from an Indian case of kala azar and was kindly provided by Professor S ADLER in January, 1940 Sub-cultures were injected intraperitoneally into hamsters and when a suitable infection had developed subsequent passages were made by inoculation of infected spleen material

Preparation and counting of smears Dab preparations (contact impressions) were prepared from spleen fragments, by application of a freshly cut surface to a clean glass slide after removal of superfluous blood About thirty to forty dabs were made on each slide to provide a good selection for counting The preparations were dried in air for an hour or two fixed for 2 minutes in absolute alcohol and stained for 2 hours in Giemsa stain diluted with water adjusted to pH 7 4 with lithium carbonate. This procedure gave clean preparations free from stain deposit and uniform in tint. Counts were made under the 1/12 inch oil immersion objective, using a squared eyepiece A green filter (Wratten B) was found useful to avoid eye-strain.

The thicker areas of contact preparations are unsuitable for counting, and some selection of fields is unavoidable In order to avoid bias in choosing the fields, the microscope was racked up so that the outlines of the spleen cell nuclei were visible, but the parasites were just out of focus Suitable fields containing thirty to seventy nuclei were selected, and the parasites focused and counted.

The disadvantages of the use of imprint preparations for quantitative work have been indicated by OSGOOD and SEAMAN (1944) with reference to the differential cell-count in bone marrow The method that these authors recommend for bone marrow is not applicable to spleen material, however, and imprint preparations appear to offer the best alternative.

RESULTS

1   *The number of nuclei and parasites to be counted*

Macrophages containing numerous parasites are found in dab sections as well as scattered organisms from ruptured cells and points

forms. The theoretical formula for variation in count due to random sampling errors cannot therefore be applied to the problem of how many nodes of parasites should be counted to give a representative result. In order to obtain a measure of the actual variation, four sets of ten determinations of parasites per 100 nuclei were made, the number of nuclei counted in each set 100 250 500 and 1,000 respectively All counts were made from the slide and all included fields from several dabs selected at random. The results for a moderate and a light infection are shown in Table I

TABLE I

OBSERVED VARIATIONS OF PARASITE COUNT IN INFECTED SPLEENS.

Hamster No.	No of Nuclei Counted	Ten Counts of Parasites per 100 Spleen-cell Nuclei										Mean (M)	Standard Deviation (σ)
61	100	85	140	47	54	71	34	12	20	56	57	58 3	33 4
	250	31	40	41	54	45	54	18	41	36	31	39 4	10 5
	500	62	43	48	52	76	4	40	46	4	36	45 4	1
	1 000	41	53	41	64	63	41	41	56	48	23	45 1	9 1
211	100	12	8	54	0·8	6 7	3·4	4·9	6 7	4 3		5·39	3 5
	250	12	4 3	6·0	11	2 1	7·6	4 9	8	8·9	10	6·96	3 6
	500	44	85	84	4·6	3·6	68	7 2	2 2	53	54	5 19	1 57
	1 000	48	80	51	62	40	7·6	7 3	6	7 3	45	5·44	1 43

The table shows that with the moderate infection (Hamster 61), a count of 250 to 500 nuclei gives a reasonable estimate of the degree of infection, the additional effort required to count 1 000 nuclei is not repaid by any improvement in accuracy With the lighter infection (Hamster 211), 500 to 1 000 nuclei must be counted before the results become uniform. It is apparent that it is useless to record the parasite count to more than two significant figures.

2. *The variation of count between dabs on the same slide and between different positions in the same spleen.*

Four infected hamsters were killed, and the spleens removed. Each spleen was cut transversely into ten approximately equal fragments and dab preparations made from the cut surfaces. In each of five dabs at every level, 500 nuclei and the accompanying parasites were counted. An analysis of variance of the fifty counts for each spleen was made and the results are shown in Table II. The scatter of the individual counts for Hamsters 59 and 211 is shown in Fig 1 (p. 156)

TABLE II

ANALYSIS OF VARIANCE OF FIVE COUNTS OF 500 SPLEEN-CELL NUCLEI AND THE ACCOMPANYING
PARASITES AT TEN LEVELS IN THE SPLEENS OF INFECTED HAMSTERS.

Hamster No	Item	Sum of Squares (S.S)	Degrees of Freedom (N)	Mean Square (S.S /N)	Variance Ratio	Probability
59	Between levels	1,342·48	9	149·11	1·56	0·2 (not significant)
	„ dabs	364·23	4	91·00	1·05	>0·2 („ „)
	Interaction	3,438·5*	36	95·53		
	Total	5 145·23	49			
61	Between levels	1 454·32	9	161·59	2·48	0·05 (significant)
	„ dabs	513·32	4	128·33	1·97	0·2 (not significant)
	Interaction	2,342·28	36	65·06		
	Total	4,309·92	49			
56	Between levels	207·42	9	22·49	1·04	>0·2 (not significant)
	„ dabs	7·37	4	18·09	1·30	>0·2 („ „)
	Interaction	845·41	36	23·48		
	Total	1 120·20	49			
11	Between levels	95·45	9	10·61	2·52	0·05 (significant)
	„ dabs	12·84	4	3·21	1·31	>0·2 (not significant)
	Interaction	151·64	36	4·21		
	Total	259·93	49			

These results show that the distribution of parasites in the spleens of
Hamsters 59 and 56 is homogeneous, but that in Hamsters 61 and 211 the
differences of count between levels are statistically significant. The significance
is not of a very high order as the probability against the observed variations
being due to random errors of sampling is about nineteen to one.

A further six animals were examined in rather less detail, 1 000 spleen-cell
nuclei being counted in fields from all parts of a slide, at ten levels in the spleen
of each animal. The results are summarized in Table III The four hamsters
previously examined are also included the figures for 1 000 nuclei in these
animals were obtained by selection of field-counts from the data already col-
lected with the aid of a table of random numbers (FISHER and YATES 1938)

As would be expected from the method of counting the figures for the
lightly infected animals 375 56 and 211 are more variable than the rest. The
variation could be reduced by the counting of a greater number of nuclei,

TABLE III.

PARASITE COUNTS BASED ON 1000 NUCLEI AT TEN LEVELS IN THE SPLEENS OF INFECTED HAMSTERS

Hamster No.	Mean ± 10 Counts (M)	Standard Deviation (σ)	M/σ	M − 3σ
508	149	10	14 9	122
360	61 8	1 3	8·0	49
89	59·4	8 3	7 1	34 3
343	54 3	9·4	5 9	46 9
61	49·6	8	9	21·0
350	13 9	1 4	0 9	97
46*	14 1	I 5	9 4	96
375	10 9	3·6	3·0	0 1
34	16·8	3 7	2·9	0
11	3·69	1 1	3 3	0·4

FIG. 1.—The scatter of individual parasite counts in the spleens of Hamsters 59 and 211. The counts are plotted on a logarithmic scale to give a fair idea of their relative magnitudes.

but for the purpose of routine tests animals with a count of less than ten parasites per 100 nuclei are best avoided owing to the extra labour involved In the more heavily infected hamsters the parasites are distributed fairly evenly through the spleens, and the differences in Hamster 61 revealed by the variance analysis do not appear to be important If three times the standard deviation is subtracted from the mean in each of the first seven animals in Table III the remainder is about one-half of the mean This indicates that an observed reduction of count to one half of the original value by drug action would be expected to be due to random sampling only once in 1 000 times

3 *The development of infection in control animals*

The behaviour of the infection in control animals was studied at time intervals corresponding with those to be used in therapeutic tests. The animals were anaesthetized with ether and spleen dabs prepared from biopsied material. The results of counts made at 1 and 3 weeks after the initial biopsy upon seven animals are shown in Table IV

TABLE IV

THE DEVELOPMENT OF LEISHMANIA INFECTION IN THE SPLEENS OF HAMSTERS.

Hamster No.	Parasites per 100 Spleen-cell nuclei.		
	Initial Biopsy	1 Week Later	3 Weeks Later
1*5	6	58	100
400	16	50	53
407	23	28	111
398	27	33	94
534	21	30	—
450	53	66	—
35t	15	24	—

All animals show a pronounced increase of infection even 1 week after the initial biopsy The effect of surgical interference lowers the resistance of the animals, and allows the infection to increase rapidly in intensity This is especially noticeable in Hamster 123 in which the parasite count increased tenfold in the course of a week. With this strain of leishmania the count is usually increased 1 5 to 2 times in one week after the initial operation. This being the case, if the drug merely prevents any increase in the infection observed 1 week after the first biopsy it is very probable that the treatment has had an effect. A reduction of count to one-half of the initial value would have a very high significance, far outweighing errors of random sampling It is of interest to note that the estimates of infection before treatment made by FULTON (1944) who allowed a week for recovery from the first operation before beginning drug treatment, may be appreciably lower than the true values

4 The effect of drug action.

There remains the possibility that the effect of a drug upon the micro may not be uniform in all parts of the spleen This problem was investigat by removing a strip from the entire lateral edge of the spleen of an anaesthetis infected animal, about one third to one half of the total spleen volume bei excised. The larger blood vessels entering the spleen via the mesentery wa carefully avoided, and haemorrhage from the cut surface was not extensi
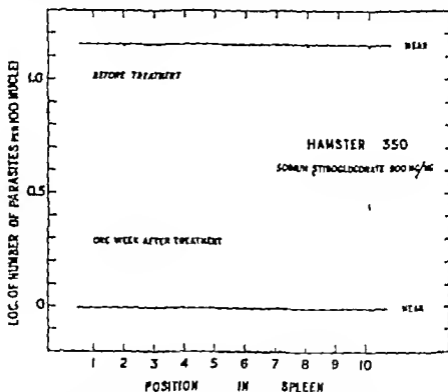
FIG. 2.—The effect of a single subcutaneous dose of sodium stibogluconate up parasite counts in the spleen of Hamster 350.

The excised strip of spleen was cut transversely into ten pieces, and dabs pared from each level. The next day a single subcutaneous dose of drug given, and 1 week later the animal was killed. The remainder of the spleen w removed, divided transversely into ten pieces corresponding with those prepar from the excised strip and dabs prepared from each level. Counts were ma for each of the ten levels in the spleen before and after treatment. Four hamst were examined in this way and the results are shown in Table V The individ counts for one of them are shown graphically in Fig 2. The means of th counts before treatment have already been recorded in Table III.

The results show clearly that the effect of drug action is uniform throughout the spleen, and that a count made from any part of the organ after treatment is a fair sample of the whole. Statistical treatment of the results shows that for all the animals examined there is a highly significant difference in count before and after treatment. A value of t of two and over is considered to

TABLE V

THE EFFECT OF DRUG TREATMENT UPON THE PARASITE COUNTS AT TEN LEVELS IN THE SPLEENS OF INFECTED HAMSTERS. (ALL COUNTS MADE UPON 1 000 NUCLEI)

Hamster No	Drug Treatment		Mean of 10 Counts (M)	Standard Error of M (s).	$t = \dfrac{M_1 - M_2}{\sqrt{s_1^2 + s_2^2}}$
350	Sodium stibogluconate 500 mg /kg	Before treatment	14 1	0 49	27
		After treatment	1·0	0 10	
402	Sodium stibogluconate 250 mg /kg	Before treatment	13 0	0 46	23
		After treatment	2 7	0 1	
375	Neostam 250 mg /kg	Before treatment	10·0	1 15	9·0
		After treatment	0 3	0·06	
363	Neostibosan 165 mg /kg.	Before treatment	51 5	1 00	0 7
		After treatment	21 8	0 93	

be significant. The action of single doses of drugs upon leishmania infections can therefore be assessed by the effect upon the parasite count in the spleen.

SUMMARY AND CONCLUSIONS

1 In infected hamster spleens containing fifty and over leishmania parasites per 100 spleen-cell nuclei, a fair estimate of the degree of infection can be obtained by counting 250 to 500 nuclei. For infections of less than ten parasites per 100 nuclei 500 to 1 000 nuclei must be counted. Lightly infected animals are unsuitable for accurate therapeutic tests.

2. The distribution of organisms in the spleen assessed by parasite counts in imprint preparations is not grossly uneven

3 Infected control animals show a steady increase of infection which may be very rapid after spleen biopsy

4 Single subcutaneous doses of organic antimonials are shown to be
a measurable effect upon the parasite count in the spleen, and drug action
uniform throughout the organ.

5 These observations form a sound basis upon which a therapeutic
potency test may be designed.

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A CASE OF BLACKWATER FEVER IN AN AFRICAN GIRL

BY

J O SHIRCORE c.m.g. m d. m.r.c.p.e.

Medical Officer Karonga Nyasaland

The case is that of an African girl 12 years old—both parents Ahenga—who was born at Dar es-Salaam Tanganyika Territory and the present attack is the third. The first occurred 5 years ago at Tukuyu a small township 38 miles north of Lake Nyasa the second at Dar-es-Salaam, 3 years ago the third at Mwambetania a village about 2 miles south of Karonga the head quarters of the North Nyasa District Nyasaland on the evening of the 23rd May 1944 and the patient walked to hospital from her home during the forenoon of the 24th.

The first I saw of her was sitting upright on the edge of the bed, while her temperature was being taken—it was 104 F There was no sign of restlessness or exhaustion neither then nor during the whole course of the illness, and her appearance was that of a patient suffering from a trivial ailment The most she complained of was a headache and slight pain in the loins for a few days. The urine, on admission, and for 4 days was porter coloured and, thereafter until the 16 44, was of a light port wine tint. There was a good deal of deposit

A few coarse rings were observed which I took as quartan malaria, and in all 1 875 grammes of mepacrine which included one intramuscular injection of 0 375 gramme was given in 4 days

The temperature fell by lysis on the 4th day to 99 4° F and then rose gradually to 102 4° F on the 7th. It was 102 F on the 8th and fell again by lysis to normal on the 12th day Thereafter the temperature only rose to 99 0° F on four occasions, and she was discharged on the 24th day of June.

Microscopic examination of the stained urinary deposit revealed a heavy bacterial infection the outstanding being a streptococcus in chains of 25 to 30 cocci

Apart from the mepacrine, the treatment broadly conformed to Hearsey s method, and later, as the urine was loaded with uric acid crystals potassium citras was given.

In view however of the bacterial infection, sulphapyridine, 0·5 gram was given, t.i.d. on 28.5.44 and the day after with no visible effect the blackwater but while the bacteria as a whole were much diminished number the streptococci were still largely in evidence

Sulphathiazole was substituted on the 1st June, and within 3 hours blackwater cleared, with an occasional pink tinge during the next 24 — and thereafter gradually assumed the normal colour over a period of — with the urine free from streptococci.

Perhaps, at this juncture, it might not be out of place to state that this case was the 103rd example of blackwater fever of those which have been under my care and the first in an African.

The 102 previous cases all occurred between the years 1908 to —. Amongst them there were a few civilian Europeans and Asiatics. Two — former my first cases, occurred at Blantyre during the last few months 1908 and were investigated by YORKE—a member of the Liverpool School Commission on Blackwater Fever to Nyasaland the rest were Indian troops of the Expeditionary Force drafted to East Africa during the last war. Of these cases, fourteen died.

In this connection, all relevant records were forwarded to the civil — military authorities presumably therefore, they are still available

Heavey s treatment—modified, when necessary by the substitution — potassium citras for sodium bicarbonate, and a suspension of mercury adopted as basic, and symptomatic treatment, including the active, and — exhibition of brandy champagne, meat extracts, such as Valentine s juice chicken and other essences, in two teaspoonful doses—i.e brandy — essence—every hour when the patient was in a weak and sinking condition Adrenalin was also found useful occasionally In my belief more than — case was saved by this form of supporting treatment.

It is deplorable that of all the above cases—except the present one — of the urinary deposits was stained and examined for bacteria and, of as a consequence, it is now impossible to correlate the present findings any previous example nor can I recollect the record of any bacterial examination of the urine and blood in the literature of blackwater fever

The bearing of a haemolytic streptococcal infection of the renal pelvis with the probability of a concurrent septicaemia, on the aetiology and mechanism of blackwater certainly demands close investigation, by cultural methods including blood culture, for it is not beyond the realms of possibility that an organism of this nature might be a contributory factor if not the cause, blackwater fever in the malarial subject.

There is no bacteriological laboratory at Karonga, and no research, therefore could be undertaken.*

*A cablegram received later from Dr. SHIRCORE reads   Strong indication coccus in deposit cause and sulphathiazole specific blackwater "—ED.

CORRESPONDENCE

RHODESIAN SLEEPING SICKNESS

To the Editor, Transactions *of the Royal Society of Tropical Medicine and Hygiene*

SIR,

I have read with much interest Dr MacKichan's paper on *T rhodesiense* [*] and having some acquaintance with the country and the problems covered in this report, perhaps you will allow me to record a few comments.

The case is certainly strong against *G pallidipes* as the main vector in this epidemic. This fly feeds mainly on game and so has free access to the polymorphic trypanosomes, but in both Eastern and Northern Uganda it bites man readily. *G palpalis* on the other hand, draws largely on reptiles, in which these parasites are not found in nature. Investigations carried out at the Human Trypanosomiasis Institute at Entebbe showed that at all events under local laboratory conditions, *G morsitans* also a game feeding species, is a better transmitter of polymorphic trypanosomes than is *G palpalis*. Also that *T rhodesiense* is, in general, more readily transmissible cyclically by tsetse than is *T gambiense*. It was found that many strains of *T gambiense* particularly those recovered from cases of long standing possessed a very low transmissibility some being unable to complete the cycle in *G palpalis* by reaching the salivary glands of the fly. In this connection it is interesting to note that of sixty five wild *G pallidipes* from the escarpment above Lake Albert, 3 per cent. were found on dissection to have heavy gland infections (DUKE, 1916). This is an unusually high figure for polymorphic trypanosomes in wild tsetse of any species.

The conclusion that the trypanosome in this epidemic was introduced from N.E. Tanganyika in natives visiting Uganda in search of work, seems to be well founded. As will be seen in a moment, this has actually happened in the past.

Discussing the results of inoculating volunteers, the author suggests that the failures were due to the strain being either 'an innocuous *T brucei* or 'a non infective *T rhodesiense* Surely this is a distinction without a difference. There is however, another cogent factor studied for several years

[*] MACKICHAN I W (1944) Rhodesian sleeping sickness in Eastern Uganda. *Trans. R. Soc trop Med Hyg* 38 49

at the H T L namely variations in individual human resistance. This is an aspect of the aetiology of the epidemic that should not be overlooked.

There is one statement in Dr MacKichan's paper (page 55) which both puzzling and misleading. It reads "This is apparently the first that this strain has been isolated in Uganda [by this strain" be presumed means *T rhodesiense*] there is a record of a previous case in the Western Province being diagnosed as of the *T rhodesiense* type, but there is no evidence that annual inoculation was performed. It is possible that this sentence relates to cases detected subsequently to 1935 when the H T I was closed down and I lost intimate touch with developments in Uganda. But here are some relevant facts from the literature of previous years

(1) In December 1929 three cases of sleeping sickness, each of which had proved resistant to a full course of arsenic, were sent to the H.T.I. for the West Nile area of Uganda. All three strains were very thoroughly investigated at the laboratory the examination including behaviour in small morphology reaction to drugs, cyclical development in *G palpalis* and other reactions. Two of the strains proved to be arsenic fast *T gambiense*. The third was a typical *T rhodesiense* (DUKE, 1939)

(2) In August, 1932, several cases of a very acute form of human trypanosomiasis were found near Kampala, the native capital of Uganda. One at least of the sufferers was picked up comatose by the roadside. The trypanosome was found to be *T rhodesiense* and gave the typical reactions of the species, both in the red cell adhesion test and in laboratory animals. The patients were natives of the Buzinza country over the Tanganyika border a district west of Mwanza with a frontage on Lake Victoria of considerable extent (DUKE, 1932).

The *final Report of the League of Nations International Commission on Human Trypanosomiasis* (1928) records the isolation of a solitary case of *rhodesiense* at Bambu, near Kilo, to the west of Lake Albert, in June, 1926, just over the Uganda border The ability of a polymorphic trypanosome to invade man is the algebraic sum of man s resistance and the inherent adaptability of the trypanosome People differ in their resistance and, as with other diseases no doubt the resistance of the individual will vary with his general health Natives coming into Uganda from the west were often in my day in very poor physical condition on arrival. It is worth remembering also that where there are acute cases with many parasites in the peripheral blood direct transmission is liable to intervene in spreading the trypanosome.

In 1922, Dr GRIFFIN of the Uganda Medical Service, made a careful survey of the Eastern Province endemic areas. Again, in 1926, Dr VAN HOOF of the League of Nations Commission, spent several months in the same region. Their reports showed that the disease, then of the *gambiense* type, was kept alive mainly by small and unobtrusive foci of *G palpalis* at places of popular resort such as markets and watering and canoe landing places.

Uganda has indeed had her full measure of epidemic trypanosomiasis. The authorities and the author of this report are to be congratulated on the thorough and successful way this latest infliction has been handled. Not only has the outbreak been speedily analysed and brought under control; in addition *G. pallidipes*, hitherto regarded as a potential rather than an actual danger to man himself, must now join the roll of convicted criminals of the genus *Glossina* as an efficient carrier of sleeping sickness.

I am, etc.

H. L. DUKE

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HEAT EFFECTS

To the Editor TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene*

SIR,

Air Commodore MORTON'S valuable paper on "Heat effects in British Service personnel in Iraq" * has just arrived here. He complains that the subject of biochemistry in heat effects is in a state of flux, whatever his means. Further on he states that 'what the clinician wants is a method of treatment which will safely restore the disordered metabolism. A treatment which is too specific, for example alkalies to treat acidosis or ammonium chloride or alkalosis is too dangerous *unless the services of a well-equipped laboratory are at hand*' (Italics are mine.)

There is pretty general agreement that the services of a well equipped laboratory should always be at hand, particularly in camps or other human aggregations big enough to have a medical organization in tropical countries. It is time the clinicians learnt to work *with* a laboratory to put a true value on its findings and to be guided by its help in the treatment of conditions in which biochemical findings are essential, e.g. diabetes, Addison's disease, myxoedema, nephritis, and even heatstroke and heat exhaustion!

The disordered metabolism' can be restored by salt therapy—as Air Commodore MORTON states—with plenty of water and bed rest *in cool surroundings*.

Most of the troubles experienced by the clinicians come from attempting to treat these cases in *hot rooms*. This 'warm therapy' for cases of heat

* MORTON T. C. (1944) Heat effects in British Service personnel in Iraq. *Trans. R. Soc. trop. Med. Hyg.*, **37** 347.

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

OPENING MEETING

of the Thirty-eighth Session of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 19th October, 1944, at 3 p.m.

THE PRESIDENT,

SIR HAROLD SCOTT, K.C.M G M.D F.R.C.P F R.S.E.

in the Chair

PAPER

SPRAY-KILLING OF MOSQUITOES IN HOUSES— A CONTRIBUTION TO MALARIA CONTROL ON THE GOLD COAST

BY

L G EDDEY M.B., CH.B D T.M & R *
Colonial Medical Service Gold Coast

CONTENTS

A

1 —INTRODUCTION

At a critical period in 1941 war strategy suddenly imposed upon Takoradi, a harbour and airport centre in the Gold Coast Colony, much activity of importance. Almost overnight, large numbers of European personnel arrived to augment its scanty non-native population. Few of the new arrivals could claim any previous acquaintance with tropical Africa and fewer still with the hyperendemic type of malarial zone in which they found themselves.

Despite attempts to improvise satisfactory housing conditions the monthly morbidity rate reached the high figure of 200·5 per mille during the early rainy period of 1941. Analysis showed that malaria alone was responsible for a monthly rate of 216·8 per mille. If only to relieve the man power strain the early institution of control measures became imperative. Strict orders were given respecting the taking of prophylactic drugs. Such precautionary measures as the compulsory wearing of mosquito boots and long-sleeved shirts were introduced. Screening materials for the eventual protection of all quarters were requisitioned. Comprehensive surveys were put in hand to determine the nature of the local swamp areas and devise means for effecting their anti-larval control.

It was realized however that, pending the completion of anti larval measures, houses in the neighbouring African townships would not only serve as day-time resting places for vector anophelines but would constitute their principal site of infection. This meant that, despite screened quarters and the practice of personal prophylaxis, susceptible personnel would, for some considerable time, run the risk of contracting malaria when visiting the township areas.

A proposal to declare these areas out of bounds was deemed impracticable. As an alternative Squadron Leader C. J. Hackett R.A.F. suggested, in January 1942, that an attempt be made to reduce the number of potentially infective anophelines in the townships by the adoption of large scale insecticidal spraying measures. This suggestion was supported by Dr. J. Balfour Kirk, Director of Medical Services Gold Coast whose experience in Mauritius had shown that spray killing measures very soon gained full co-operation from the people concerned. The pooling of Service and civil resources having been agreed upon, provision was made for the treatment of all native habitations in the danger areas using the twice weekly or more spraying frequency recommended by Covell (1941) as appropriate where anopheline infection rates are high. There followed a period of experimental spraying to determine the methods and labour organization which could best be employed. It is the conduct of the spray killing measures practised throughout the subsequent fully organized period, November 1942, to November 1943 which is now described.

II.—Areas Sprayed

The areas to be dealt with comprised the centralized portion of Takoradi township mid period population 10,505 and a group of coastline village sections total mid period population 7 162, extending into the neighbouring township of Sekondi. Of the mid period total of 1 212 houses and 7 050 rooms contained in the combined areas 618 houses and 3 795 rooms were located in Takoradi township. Nearly all rooms possessed solid walls as also close fitting doors and louvred or closed batten windows. There were ceilings present in some 50 per cent, of cases. Little difficulty was experienced therefore in creating a temporarily effective insecticide concentration in each room on closure.

III.—Labour Organization

For purposes of administrative convenience the areas treated were sub divided into nine zones of approximately equal size. The whereabouts of each zone is denoted alphabetically in the map forming Fig. I page 170.

Trained African clerks designated statisticians measured the rooms in all zones and calculated their cubic capacity so that the amounts of spraying done might be interpreted in terms of standardized units of 1 000 cubic feet each, a room factor being unsuitable since scarcely any two rooms were identical in size.

To each zone was allocated a company comprising one statistician, one semi literate headman and ten labourers. The procedure was that a team of six labourers under the headman sprayed two rooms at a time. Two sprayers worked inside and one outside each room simultaneously the last mentioned being employed to create a barrier zone of insecticide over the previously closed window and door apertures. After a 15-minute interval the two sprayed rooms

were entered by the remaining four labourers. Two labourers swept each room under the supervision of the   statistician   all dead and stupefied mosquitoes being collected and their numbers recorded

TAKORADI MALARIA CONTROL SCHEME 1942/3
Plan of Area showing Spray Killing Zones

Fig 1

	KEY TO ZONES
A	Assaning
B	Essami Extension
	Efua Nkwanta
	Park Montcampo
C	Nkantampo
D	Takoradi Zongo & Township
E	Takoradi Township
F	
G	(1) Lower New Takoradi
	(2) Upper New Takoradi
	(3) Fishel Village
H	Takoradi Township
I	
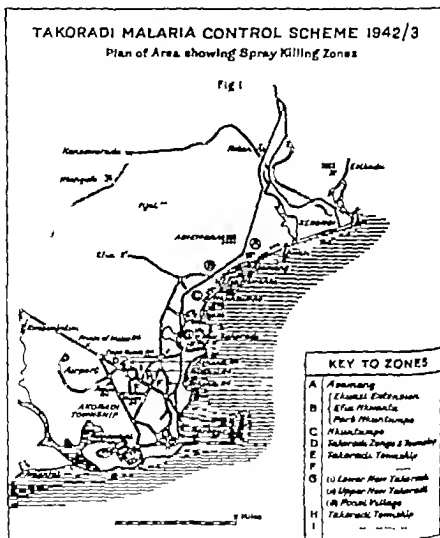
Two Africans were appointed as   Senior Statisticians   to supervise township and village sections between them and were, in turn responsible European Superintendent exercising general supervision with the assistance an African Clerk/Timekeeper

IV.—PUBLIC CONSIDERATIONS

No active opposition was encountered among the occupants of rooms sprayed. There was in fact ample oral evidence that as was the case in villages of South India when first sprayed by RUSSELL and KNIPE (1939) a marked lessening of mosquito nuisance was noted and appreciated by the public.

Unfortunately regular spraying access to a number of rooms was unobtainable by reason of the fact that certain unmarried types of tenant kept their rooms locked whilst absent at work. It must be assumed, therefore that each spraying left sufficient live female anophelines in locked rooms to maintain some potentially infective foci in each zone. However the proportion of such locked rooms fell rapidly from the initial figures of 17 5 and 15 7 per cent. in November and December 1942, respectively to an average of 9 74 per cent. for the months of 1943. It is considered that this reduction was largely due to the development of a greater willingness to co-operate on the part of the public once appreciation of the benefits of spray-killing became general.

V.—RAINFALL AND MOSQUITO PREVALENCE.

(1) *All Species.*—The direct relationship between mosquito prevalence and rainfall is shown in Table I which indicates also that a total of 528,254 room

TABLE I

Month.	Rainfall.		Takoradi Township		Village Areas.	
1942-43	Amount in Inches.	Number of Wet Days.	Number of Units Sprayed and Swept.	Average Number of Destroyed Mosquitoes Collected per 1 000 Units per Spraying.	Number of Units Sprayed and Swept.	Average Number of Destroyed Mosquitoes Collected per 1 000 Units per Spraying
November	1 18	10	25,204	2˙ 80	13 ˙47	26 90
December	3 39	6	22 907	33 50	16,240	13 ˙70
January	0 45	4	24 922	50 50	18 807	7˙ ˙0
February	2 12	4	˙7 333	49-50	18 144	11-60
March	3-60	7	29,243	49-00	20 ˙89	15 50
April	8 ˙0	15	30 497	45 40	19 ˙54	30-40
May	18 34	18	32,095	˙0 50	18 695	25-00
June	6-96	18	31 103	3 4-00	19 852	60 80
July	4-0˙	11	35 025	134-50	˙0,337	85 49
August	1 22	9	33 327	53-00	19 534	14 80
September	1-09	14	32 ˙53	21 ˙0	19,359	3-60
October	7˙ 2	3	32,664	3-0˙	18,248	3 80
November	3 ˙6	13	36 ˙4˙	3 ˙9	20,50˙	8-04
Totals	6...08	145	393 818		˙43,993	

were entered by the remaining four labourers. Two labourers swept each one under the supervision of the " statistician," all dead and stupefied mosquitoes being collected and their numbers recorded.

TAKORADI MALARIA CONTROL SCHEME 1942/3
Plan of Area showing Spray Killing Zones

Fig 1

KEY TO ZONES

A	Assamang
B	(1) Eikwesi Extension
	(2) Fia Mwanza
	(3) Park Mkantumpa
C	Mkantumpa
D	Takoradi Zongo & Township
E	Takoradi Township
F	
G	(1) Lower New Takoradi
	(2) Upper New Takoradi
	(3) Fishel Village
H	Takoradi Township
I	

Two Africans were appointed as Senior Statisticians to supervise the township and village sections between them and were, in turn, responsible to a European Superintendent exercising general supervision with the assistance of an African Clerk/Timekeeper

Regarding the use of actual amounts of rainfall as the basis for these monthly comparisons it may be added that these amounts were correlated closely with both the trend of the monthly totals of wet days and of the monthly average rainfall per wet day.

VI.—ENTOMOLOGY

Much of the entomological data which follows is drawn from work done in this area by Capt. P. F. MATTINGLEY and Lieut. J. D. ROBERTSON R.A.M.C. to both of whom I am greatly indebted.

GRAPH: DESTROYED MOSQUITOES COLLECTED PER 1000 UNITS PER SPRAYING
Fig II
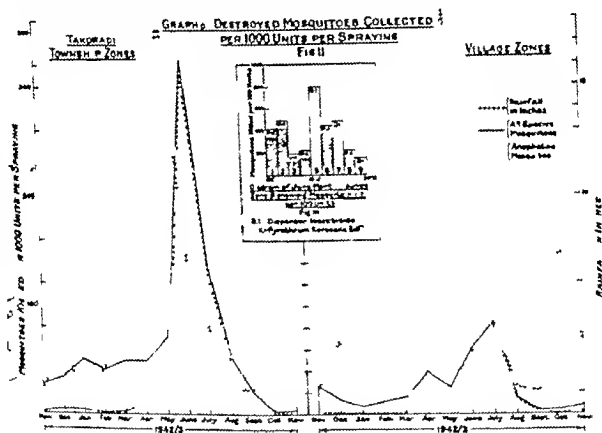
Anopheles gambiae the principal local vector of malaria, persisted throughout the spray period as the predominating anopheline species killed. *A. funestus* also a vector species was recorded occasionally and such other anophelines as *A. pharoensis* and *A. paludis* only very rarely. The identifications given in Table III were obtained in respect of batches of killed anophelines selected at random during the months mentioned. Dissection of 100 specimens of female *A. gambiae* selected at random during July 1943 revealed an infection rate of 3 per cent.

Aedes irritans a crabhole breeder predominated among the culicines recorded. Included in the large variety of occasional culicines were *Culex*

*nebulosus C rima Uranotaenia annulata Afd punctothoracis C thalassius &
Aed aegypti* the last mentioned being a known vector and the second the
potential vector of yellow fever infection.

VII —INSECTICIDAL METHODS.

Initially all zones were treated with a standard 1 160 cresol-kerose
mixture (i.e. 1 ounce of added cresol per gallon of kerosene) which was dis-
persed from the intermittent " flit-gun " type of domestic hand pump, this
combination of insecticidal materials being the only one then available in suffi-
cient quantity Subsequently as supplies came forward, selected zones were
treated with either pyrethrum dusted dry kerosene extract of pyrethrum or
pyrethrum aerosol The differing results obtained are thought worthy of
detailed comment.

TABLE III

| | December 1942. | | January 1943 | | July 1943 | |
Species	No.	Percentage	No.	Percentage	No.	Percentage
A gambiae—female	182	91	299	94 75	197	85 65
—male	12	6	1	3 93	43	12 17
A funestus—female	6	3		0 66	4	1 74
—male	—	—		—	1	0 44
Other anophelines—						
female	—	—		0 66	—	—
male	—	—		—	—	—
Totals	200	100	303	100%	230	100%

(I) DRY PYRETHRUM.

A first departure from the cresol-kerosene basis was made in respect of
Zone D—pulverized pyrethrum, dusted dry both from Waldron distributors
and hand-operated bellows-type paris green sprayers, being substituted through-
out the period December 1942 to March, 1943.

The pyrethrum had been flown to the Gold Coast from Kenya and, pending
use remained in its original paper lined sack containers in a dry well ventilated
storeroom. Dr S A B BLACK (1943) working with pyrethrum under similar
conditions in Lagos, Nigeria, has recorded, in a personal communication, the
opinion that the powder as used had lost about half its original pyrethrin content
He supports his opinion with the statement that powder from fresh Kenya
pyrethrum flowers contains 1 3 per cent. total pyrethrins whereas the powder
as used in Lagos was reported on by the Nigerian Government Chemist as
follows :—

Analysis gave pyrethrin I 0 25 per cent. pyrethrin II 0 44 per cent It is probable that there has been a loss of 50 per cent. in the Pyrethrin I content. According to C B GRADINGER and C. S. CORI (*Ind Eng Chem.*, 1932, 901) freshly ground pyrethrum flowers in containers of different kinds, lose from 30 to 44 per cent. of pyrethrins within a year

Despite this diminution of strength good kills were obtained as Table IV demonstrates. It was found that so small an average amount of the powder as 0 35 ounce by weight per unit of 1 000 cubic feet produced a sufficiently unpleasant atmosphere to necessitate withdrawal of the sprayers The routine dosage was based on this criterion and proved very lethal in spite of its smallness compared with the 1 to 2 ounce dosage per 1 000 cubic feet used when such powerful distributing agencies as Cyanogas dust pumps are available

Having regard to the repellent effects of dusted pyrethrum demonstrated by SYMES McMAHON and HADDOW (1942) a departure was made from routine

TABLE IV

Month, 1942-43	Total Units Sprayed and Swept.	Number of Destroyed Mosquitoes Collected.	Number of Spraying Rounds.	Average Number Destroyed Mosquitoes Collected per 1 000 Units per Spraying		Spraying Method in Zone D
				Zone D	Takoradi Township	
November	5 658	1,388	8½	23 8	2ⁿ 8	C K × 2
December	3 499	793	4½	50 4	33 8	P D × 1
January	3 208	702	4	54 ⁿ	50 8	
February	5 966	1 169	8	24 3	40·6	P D × 2
March	6 789	2 951	9	48 3	49·0	

Note —C.K. = Cresol-kerosene   P D = Pyrethrum Dusting   × 1 = once weekly
× = twice weekly   a "Round" is one circuit of the complete zone

procedure in that no sweeping up of the sprayed rooms was undertaken so as not to disturb the deposited dust The collection of knocked-down specimens was somewhat handicapped by this arrangement and it is possible that a small percentage of mosquitoes destroyed escaped observation.

As indicated in Table IV a variation in spraying frequency was also introduced in a broad attempt to ascertain whether in view of the repellent properties of the dust, once weekly or twice weekly treatment could be considered the more efficacious in practice The general indications are that twice weekly treatment tended to result in a lowered kill per spraying thus suggesting that some repellent effects may have persisted over the half week period But, in view of the disparity in each of the two categories between the monthly findings themselves it is considered that the results obtained have not the value which carefully controlled laboratory experiments conducted on similar lines, might

yield. As is to be expected in so large and primitive a field, results were probably vitiated to some extent both by householders attempting to sweep up the powder from its more obvious resting places and by the occurrence of localized breaking in Zone D out of proportion to that occurring in the other zones of the township.

Possible repellent effects apart, all mosquitoes observed in the room immediately after spraying were either dead or stupefied and householders reported a sustained absence of cockroach pests throughout the months of treatment. In view however of the high rate of pyrethrum consumption using the dry of the kerosene extracted form, general use of the dry material was rendered impracticable both by reason of limitation of supplies and high costs.

(2) PYRETHRUM-KEROSENE.

Throughout the period April to November 1943 which was inclusive both the major and minor rainy seasons for the year a standardized pyrethrum-kerosene preparation was used in all Takoradi township zones. In preparing this insecticide half a pound of the dry powder was added to each gallon of ordinary commercial kerosene and a double extraction effected over a total period of 48 hours. It may be mentioned in passing that the kerosene-extracted residue which remained after final separation of the liquid product proved an effective anti-larval agent when applied to collections of standing water.

(3) CRESOL-KEROSENE.

The original 1:160 cresol-kerosene preparation was used throughout the April to November 1943, period in all the village zones. The relatively long term data both as to work capacity and insecticide consumption, resulting from the extended use of cresol-kerosene and pyrethrum kerosene in the village and township areas respectively are compared in Table V. Item (d) of this table demonstrates that the average amount of cresol-kerosene used per unit exceeded the pyrethrum kerosene amount by 22.8 per cent. Since the sole criterion in respect of insecticide quantities used per unit was the degree of discomfort produced in the upper respiratory passages of unprotected spray personnel, it is clear that if it were desired to use both preparations in equal amounts per unit pyrethrum kerosene would, from the sprayer's standpoint, produce the more pleasant medium in which to work.

The capacity of the average room in Takoradi township was found to be 1,288.6 cubic feet, i.e. 1.2886 units, and in the village areas 1,005.9 cubic feet, i.e. 1.0059 units. It will be noted therefore that each sprayer in Takoradi township area dealt with a daily average of 44.62 units, equivalent to 34.6 rooms, whereas in the village areas only 34.60 units, equivalent to 31.57 rooms were dealt with per day the approximate duration of a working day being 6½ hours. This difference is accounted for partly by the fact that the cresol kerosene used in the villages required a greater spraying time per unit and

a greater amount of the insecticide had to be pumped per unit than when pyrethrum-kerosene was employed The principal reason for the difference is, however, that portions of the villages were somewhat scattered as compared to the township and a greater proportion of time was utilized in moving from one house group to another

It was the common experience in both areas that progress from room to room depended as much on the willingness of room occupants to facilitate access and the temporary removal of contained water and foodstuffs as on the actual time required by the spraying staff to effect closure and the dispersal of insecticide

TABLE V

Spraying Data.	Pyrethrum Kerosene.	Cresol Kerosene.
(a) Average units sprayed per company per day	25 0	203 20
(b) Average units per sprayer per day	41·62	34·60
(c) Average units sprayed per gallon of insecticide ..	132 39	109 25
(d) Average fluid ounces of insecticide used per unit	1 21	1 47

(4) PYRETHRUM AEROSOL.

During June/July 1943 two trials were given to Westinghouse dispensers supplied from United States Army sources The dispensers were of one pound size and contained pyrethrum insecticide dissolved in liquefied freon gas which when released, produced an aerosol described by the makers as non toxic and non inflammable   Evaporation of the gas and the colloidal dispersion of the contained insecticide appeared to be both rapid and penetrating

(i) *Speed of Method* —The first trial designed to assess the relative speed of the aerosol method consisted in employing the dispensers in Zone D of Takoradi township This zone contained 99 houses with 740 rooms of township totals of 618 houses and 3 795 rooms. The estimated population of the zone at this time was 1,936 of a township total of 10 505

The dispenser rounds were alternated with rounds using the pyrethrum-kerosene solution on the lines already described. During the half-month period a total of five rounds three by dispenser and two by hand sprayer technique was completed. For the dispenser rounds a variation from the usual spraying procedure was found necessary In the first instance the rooms continued to be sprayed in pairs the company headman and a labourer dealing with one room and the statistician with a labourer dealing with the other The remaining eight boys of Zone D s company were divided into two sweeper teams of four boys each.

When the reconstituted teams became practised it was evident that, owing to the very short time required for actual dispensing the two groups of sweepers could not keep pace with the two sprayers even though four labourers per team represented a doubling of sweeper personnel. Accordingly for the third dispenser round, only the statistician and a labourer assisting were employed on the actual spraying process the nine remaining labourers being divided as three teams of three sweepers each. Not only did a speeding up result from this arrangement but there was a marked increase in efficiency due to the labourer man being left free to revert to his normal supervisory function.

The dispenser manufacturers had recommended 4 seconds spraying per 100 cubic feet of confined space. Since the average room size in Zone D was 1,500 cubic feet and the absence of ceilings in many of its one-storey buildings made closure between wall-plate and roof incomplete, a minimal exposure of 5 seconds per room was considered necessary. In practice the assisting labourer having closed all doors and windows the dispenser operator timed his release by counting " 101 102, 103 104 and 105." It was found that an exposure of not less than 6 seconds was usually obtained by this routine. That this exposure was adequate is clear from the fact that, following a quick withdrawal from the rooms and the maintenance of their closure for a period of 15 minutes thereafter all mosquitoes observed were either dead or stupefied when the rooms were reopened.

The results achieved, which are given in Fig III on page 12, include, for the sake of completeness those obtained by the use of pyrethrum-kerosene solution in Zone D during the first half of 1943. As the test period coincided with a very large increase in the local mosquito population following upon an exceptionally heavy rainfall in the preceding month, the comparison of the two types of spray killing method afforded was even more adequate than anticipated.

In the matter of spraying time the dispenser method as finally adopted, proved the more efficient since its average of 43 3 rooms equivalent to 60 1 units spent and swept per hour was 40·1 per cent. greater than the average of 30·9 rooms equivalent to 39 3 units recorded in respect of the pyrethrum-kerosene method.

Moreover this result was achieved despite the fact that by the dispenser method an average of 475·6 mosquitoes was destroyed and collected per 100 units as compared with 333 7 mosquitoes per 100 units by the pyrethrum-kerosene method, the greater kill of itself tending to make the collecting process in the dispenser method a more lengthy one than that required for its contemporary

(ii) *Use in Specific Emergency.*—The second trial, which aimed at demonstrating the effectiveness of both the dispenser and pyrethrum-kerosene method under specific emergency conditions was conducted during the period 1st to 14th July 1943 on a newly occupied housing estate sited near to Zone A. This estate was particularly suitable for the purpose in view since it stood on a virgin site where an unexpectedly heavy infestation with mosquitoes had occurred. Further it contained a large number of standardized rooms and these were arranged in identical groups to form block compounds

All the rooms chosen measured 12 by 10 by 8 ft. 9 in. high giving a cubic capacity of 1 050 cubic feet. Each room was completely ceiled and equipped with two louvred windows each measuring 3 ft. 8 in. by 2 ft. 8 in. as also one well fitting door

Forty rooms were sprayed daily in the 7 to 8.30 a m period the dispenser and hand sprayer methods being used in alternate rooms and each day's effort confined to a particular block. Approximately 5 seconds spraying was given the rooms treated by the dispenser method whilst the pyrethrum kerosene technique followed the lines already described. In all a total of 500 rooms was sprayed yielding 13,248 mosquitoes, the detailed kills being as follows —

Dispenser insecticide spraying	7 067 i e 53 34 per cent.
Pyrethrum kerosene spraying	6 181 i e 46 66 "

These results leave no doubt as to the effectiveness of both methods against high concentrations of mosquitoes. It must not be inferred however that the dispenser method demonstrates the greater killing power since it can be shown by reference to the variations given later in Table IX that the difference in the two kills could be accounted for by sampling error alone.[*] The fact that following the spraying all mosquitoes observed were either dead or stupefied suggests that the two preparations were equally effective in leaving no mosquitoes capable of flight.

(iii) *Dispenser Capacity* —The average number of rooms sprayed per dispenser on the basis of 5 seconds exposure each, was 126 equivalent to 132 3 units. Allowing for fractions of a second spent in adjustment at the beginning and end of each 5 seconds count the working life of each dispenser approximated to a total of 12 minutes.

VIII —Comparative Costs

(1) labour and insecticide

The total daily wage of a company employing one statistician at 3s 6d. one headman at 2s 4d. and ten labourers at 1s. 11d. each is 25s. Since a company's work capacity by the aerosol method over a working day of 6½ hours was found in the Zone D experiment to average 360 5 units the cost of treating 1 000 township units by this method was as follows —

Labour £3 9s 4d. dispensers (allowing 6 seconds exposure per average township room of 1 2886 units and 105 rooms per dispenser) 7 39 at 7s 6d. each, £2 15s 5d

  Total Costs by Aerosol Method £6 4s 9d

The April to November usage of pyrethrum kerosene in the Takoradi township zones utilized the services of a company for a daily average of 257 7

* Cf Raymond Pearl (1940) *Medical Biometry and Statistics* 3rd ed. Chap X and Appendix X.

units and sprayed an average of 132 3 units per gallon of insecticide. The costs per 1 000 township units were therefore —

Labour £4 17s   kerosene, at the duty free price of 13s. 4½d. per gallon 12s. 9d. pyrethrum pulv at 9d. per lb excluding air freight charges 2s. 10d. at 5s. 0½d. per lb including air freight charges, 18s. 11d

Total Costs by Pyrethrum Kerosene Method £v 12s 7d or £8 9s 3d

U ing plain kerosene as an insecticide in Zone E during February 1940 the services of a company were utilized for a daily average of 223·08 units and sprayed an average of 111 54 units per gallon of insecticide The costs per 1 000 township units were therefore —

Labour £5 12s. 1d. kerosene rates as quoted above for pyrethrum-kerosene 15s

Total Costs by Plain Kerosene Method, £6 1 8s

As to the costs of pyrethrum pulv dusting the February/March usage in Zone D utilized the services of a company for a daily average of 250·0 units and sprayed an average of 41 units per lb of insecticide. The costs per 1 000 township units were therefore —

Labour £5 pyrethrum, rates as quoted above for pyrethrum-kerosene excluding air freight charges, 19s. 4d. including air freight charges £6 2s. 1d

Total Costs by Pyrethrum Dusting Method £5 19s 4d or £11 2s 1d

The April to November usage of cresol-kerosene in the village areas utilized the services of a company for a daily average of 203 2 units and sprayed an average of 109 2 units per gallon of insecticide Estimating that more concentrated town conditions would raise the daily capacity of each company to 240 units i.e., 93 1 per cent. of their pyrethrum kerosene spraying capacity the costs per 1 000 township units would be —

Labour £5 4s. 2d. kerosene, rates as quoted above for pyrethrum kerosene, 15s. 4d. cresol, 9 16 ounces at 5s. 6d. per gallon, 4d.

Total Costs by Cresol-kerosene Method £5 19s 10d

Table VI summarizes, in order of labour expenditure, the costs of spraying per 1 000 township units. It will be noted that, quite apart from its high standard of efficiency and its non-toxic, non inflammable qualities the aerosol method is also the most economical of labour The very efficient pyrethrum kerosene method constitutes the next best in order of labour economy and is the local method of choice in the absence of aerosol supplies.

(2) COSTS IN COMMUNITY TERMS.

Unfortunately even where pyrethrum and aerosol dispensers are available their landed costs are so variable at the present time that comparison of these insecticidal methods on the basis of total costs is of only local and short-term

value. Assuming however the possession of a supply of ordinary domestic hand sprayers and the availability of sea borne supplies of pyrethrum at a landed bulk cost of 9d. per lb it is possible to set out the wet season costs incurred in Takoradi township in terms of the resident native population, thus providing an adjustable basis for estimating the probable costs of adopting the spraying method in similar communities elsewhere

TABLE VI

Spraying Agent.	Labour Costs	Insecticide Costs	Totals.		
Pyrethrum Aerosol	69 4	55 5	124 9		
Pyrethrum Kerosene	9 —	{ 15 7	112 7	Air freight charges excluded
		31 8	128 8	"	included
Pyrethrum pulvis dry	100 —	{ 19 4	119 4	"	excluded
		122 1	222 1	"	included
Cresol Kerosene	104 —	15 9	119 10		
Plain Kerosene	11° 1	15 —	12 1		

Note —All costs shown in shillings and pence

The pyrethrum kerosene spraying of a daily average of 1,288 4 units was undertaken in respect of an average of 3 795 rooms in 618 houses accommodating an average total of 10,505 persons. Since the costs excluding such special overheads as part time European supervision, amounted to 112s 7d. per 1 000 units and there was an average of 25 625 working days per month the average monthly expenditure may be represented as approximately 6s. per house, or 1¾d. per room, or 4¾d. per head of population

Given the availability of pyrethrum aerosol dispensers the monthly costs would be approximately 6s 8d. per house or 1s. 1d. per room, or 4 7d. per head of population, i.e. 10 8 per cent. more than the costs recorded for pyrethrum kerosene

Using cresol kerosene insecticide where neither pyrethrum nor aerosol dispensers are available, the monthly costs amount to 6s 5d. per house or 1s. 0¼d. per room, or 4 51d per head of population i.e., 6·44 per cent. more than those recorded for pyrethrum kerosene and 4 36 per cent. less than the percentage excess for pyrethrum aerosol.

Table VII summarizes these monthly communal costs. The slightly greater expenditures involved in using pyrethrum aerosol would almost certainly have been offset by the costs of freightage and spray-pump replacements which were incidental to the other two methods, though recent work by RUSSELL, KNIPE and SITAPATHY (1942) suggests that the hand spraying costs recorded could be materially reduced by using more efficient types of equipment than those which

have hitherto been obtainable in Takoradi   In any case it will be noted that
the spraying costs by either method amount in population terms to little more
than one penny per head per week.

The average room in the village areas had a cubic capacity equal to about
80 per cent. that of the average township room. Since smaller rooms will
require less spraying time and less insecticide the above costs should show
reduction in respect of non township areas, always provided the houses there
are not so scattered as to involve loss in transit of so much working time that
the relative saving is dissipated.

TABLE VII

Spraying Agent.	Per House	Per Room.	Per Head of Population
Pyrethrum-kerosene	0	11¾d.	4¾d
Cresol-kerosene	6 3	1 0¾	4 3¼d.
Pyrethrum aerosol	0½9	1 1	4 7¾d

IX —EVALUATION OF SPRAY KILLING AS A CONTRIBUTION TO MALARIA CONTROL

(1) MALARIA INCIDENCE IN EUROPEANS

The principal evidence as to the efficacy of the combined malaria control
measures undertaken locally is contained in Fig IV and in Table VIII. The
set out graphically and in tabulated form the 1942–43 total morbidity and
malaria incidence rates in respect of all European cases admitted to hospital
from the two main groups of Service personnel stationed in the area. No
account has been taken of illness occurring in the area among personnel in
transit.

The total malaria incidence for Group A personnel in 1943 was 42·7 per
cent. of the 1942 figure and the incidence for Group B personnel is that
amounted to only 34 2 per cent. of the 1942 figure Since the general malaria
rate in the Services throughout the Gold Coast showed only a 50 per cent. fall
in 1943 as compared with 1942, it is reasonable to suppose that whilst country-
wide measures such as screening and mepacrine prophylaxis were responsible
in part for the reduced incidence in the Takoradi area, purely local measures
exerted a valuable additional influence.

These results were achieved in spite of the fact that, in Takoradi, the year
1943 was exceptionally wet, a total of 58·67 inches of rainfall being recorded
compared to 44 56 47·81 and 48·67 inches in the years 1940 1941 and 1942
respectively.

Rainfall difficulties apart the comprehensive anti malaria drainage work

TAKORADI AREA HOSPITAL ADMISSIONS 1942/3
EUROPEAN MORBIDITY & MALARIA INCIDENCE RATES
FIG. IV
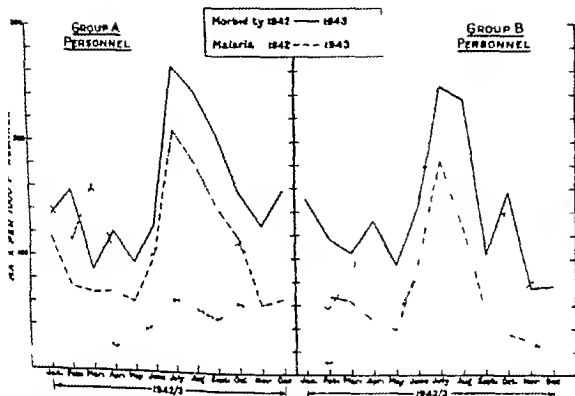
which had been organized in the meantime, though well advanced throughout the 1943 wet season, were not completed until the month of October and, even then were found to require major adjustments in respect of two main sea outfalls and several subsidiary sections.

That the drainage and oiling projects had still to attain to the full measure of their task was perhaps best evidenced in the May to June period when the monthly anopheline kill per 1 000 units per spraying increased by 870 per cent., no less than 67 000 anopheline mosquitoes being destroyed and collected in the houses of Takoradi township area during the month of June alone. It is a striking fact that no commensurate rise in the malaria incidence occurred and, with due regard to the value of personal prophylactic measures, it seems reasonable to claim that, by the early elimination of large numbers of potentially infective females among the anopheline total killed, the spray killing at this particular time played an important part in averting what might well have been a serious epidemic.

(2) MALARIA INCIDENCE IN NON EUROPEAN POPULATION

Little evidence is available as to the effect of the combined control measures on the non-European population since an attack of malaria does not normally

TABLE VIII.

EUROPEAN MORBIDITY AND MALARIA INCIDENCE RATES FOR HOSPITAL ADMISSIONS IN TAKORADI AREA.

Months	Group A Personnel.				Group B Personnel.			
	Rates per Mille.							
	Total Morbidity.		Malaria Incidence.		Total Morbidity.		Malaria Incidence.	
	1942.	1943.	1942.	1943.	1942.	1943.	1942.	1943.
January	123 10	141 50	118 20	43·68	151 8	64·2	81 4	115
February	137 50	113 00	*3 30	49 00	117 5	56 5	89 0	79
March	83·07	101·70	67 50	39 40	104·6	83·4	62 8	171
April	121 20	100 81	84 80	70·48	12..5	83·0	46 1	174
May	84 70	86 60	61 10	32 10	94·0	61 7	34 8	191
June	127 70	103 47	100 90	41·05	144·0	56 3	97·2	241
July	*63 40	12*60	209 03	64·00	210 7	83·4	183 8	173
August	242 02	113·09	140·83	53 50	226 1	75 2	134 7	211
September	201 48	101 3*	143 60	48 ·3	103·0	85·7	64 5	8*1
October	156 66	111·04	116 10	60 90	187·0	61	34·4	231
November	128 20	86 25	59 80	43 1	74·6	79 7	*8 5	231
December	156 40	134 10	64 40	61 90	77 1	94·4	16 3	391

mduce its African victim to seek medical treatment and, in any case, press of work rarely allows the out patient department staff of the District Hospital time to differentiate the purely local residents among their patients.

Verbal appreciation on the part of the native population regarding relative absence of mosquito nuisance has been mentioned already. And a sidelight on the problem was provided in February 1943 some 4 months after the start of the main spraying scheme, when, with the valued help of Capt. KIRSCHNER, R.A.M.C. parasite rates were ascertained among the locally born infants of representative village sections as recorded in Table IX.

Nkuntumpo apart from providing the lowest malaria index for all ages of infancy recorded every infant examined at 3 months and under as free from malaria parasites. This village section had shown a steady reduction in month to month totals of mosquitoes spray killed per 1 000 units per spray and was placed centrally in one of the areas receiving anti-larval attention.

The Location village group with the worst infant malaria index, was well outside the control zone. Asamang subject to spray killing measures but at to the fringe of the control zone, and Ekuase, just outside the control and spray killing zones but partially influenced by activities therein, afforded index appropriate to their intermediate status.

Accepting as does VISWANATHAN (1941) that the infant malaria index, being almost entirely unaffected by the relapse factor is the most sensitive index of the extent of transmission in a particular season then the efficacy of the combined control measures in reducing malaria infection amongst the non-European community would appear to be demonstrated.

(3) MISCELLANEOUS VALUES

(i) Supplementation of Catching Station Records

It seems worth recording that, apart from its direct benefits, the spray-killing scheme described afforded indirect assistance to the other control

TABLE IX.

Village Sections.	Infant Numbers According to Age Groups												Infant Malaria Indexes
	0-1 month.		1-3 month.		3-6 month. (Parasites present—P		6-9 month.		9-12 month. absent—A)		Totals		
	P	A	P	A	P	A	P	A	P	A	P	A	
Axumang (Zone A)	—	—	2	1	2	2	1	—	7		12	5	70·6
Ekuase	—	3	4	1	4	1	6	—	2	—	16	5	76·2
Location Group	—	1	5	1	6	—	12	—	10	—	33	2	94·3
Nkuntumpo (Zone C)	—	1	—	10	6	3	6	1	6	—	18	15	54·5

Note— "Infant Malaria Index"—Percentage of infants in whose blood malaria parasites were found.

measures being practised locally Notably so by supplementing the information furnished by the weekly captures in the various Catching Stations

These establishments yielded the usual evidence as to the existence of breeding places and the types of mosquitoes appearing in their locality Where their location coincided with one of the zones subjected to spray killing measures it was possible to supplement these data with an index of mosquito prevalence thus providing a routine series of exceptionally comprehensive pointers to local entomological conditions

(ii) Demonstration of Room to Room Variations in Mosquito Density

In the course of spray killing measures undertaken in forty identical and consecutive rooms contained in a concentrated oblong block at the housing estate referred to in Section VII (4) ii, the results given in Table X (arranged for convenience of enumeration in five columns) were obtained.

It will be observed that, with an average of twelve mosquitoes per r⊙
the individual variation ranged from none to forty three. Also that, in sele⊂⊓
rooms in this block by twos, a disparity ranging from sixty-six down to three m
quitoes could occur in adjacent pairs despite identity of size, situation, num
of occupants, method and hour of spraying It seems clear that, wherever
mosquito population tends to be considerable the variations of mos⊆
density in individual rooms are such that the results obtained from the exp⊡
mental spraying of limited numbers for purposes of any insecticide compari⊆
may prove not only unreliable but misleading

(iii) *Demonstration of Anopheline Breeding Trends.*

A further illustration of the general assistance a spray killing scheme af⊓⊂
is reflected in the table of anopheline incidence recorded (Table II). It ⅶ
be observed that the record began in November 1942, with an anopheline p
centage of 8 and ended in November 1943 with the percentage increase⌸

TABLE X.

I	II	III	IV	V
1	14	22	13	22
3	6	21	13	20
2	11	6	3	24
6	0	13	3	13
23	16	23	0	0
43	3	8	13	11
10	0	43	6	
6	3	10	10	7
93	55	101	64	109

31 29 nearly four times the original figure, despite a not dissimilar rainf⊑
the preceding 2 months of September and October was 4 30 and 7 13 inch⊂
respectively in 1942 with 1·09 and 7 72 inches in 1943 also not dissimi⊑
totals of 34 and 31 wet days for the respective 2 month periods.

That this anopheline increase was real and not the non significant conse⌸
part of the relative absence of culicine species is demonstrated by the fact th
the number of anopheline mosquitoes destroyed and collected per 1 000 ac⌸
per spraying in the village areas showed an increase from 2 15 in Novemb⊂
1942, to 2 51 in November 1943 whilst Takoradi township showed a reducti⊆
from 2 22 to only 1 18 despite all species reductions from 26·9 to 8·04 and 5¹
to 3 79 respectively

By demonstrating these relatively increased proportions of anopheline breeding despite the adoption of comprehensive control measures, the spray-killing data had the considerable value of stimulating inquiry into possible weaknesses in the local organization. The result emphasized the need for intensified oiling and vigilance in respect of all areas where formerly impenetrable swamp and overgrown valleys had been converted into easily accessible open earth drainage systems. Apart from the removal of vegetation giving consequent encouragement to *A. gambiae* breeding as pointed out by DE MEILLON (1941) the tendency of the drains themselves to retain isolated patches of seepage and to afford access to human and animal footprints is also fraught with the danger of multiplying rather than eliminating the particular types of breeding place favourable to *A. gambiae*.

V.—SUMMARY

1. An account is given of the labour organization and spraying methods used in the conduct of a large-scale anti mosquito spray killing scheme undertaken from November 1942, to November 1943 at Takoradi in the Gold Coast Colony.

2. A direct relationship between rainfall and total mosquito kills as also between rainfall and anopheline predominance, is demonstrated.

3. Insecticidal methods using cresol kerosene, pyrethrum-kerosene pyrethrum aerosol and dry pyrethrum as agents are compared in detail. It is shown that pyrethrum aerosol constitutes the insecticide of choice with pyrethrum-kerosene next best in order of labour economy.

4. The costs of applying the pyrethrum aerosol, pyrethrum kerosene and cresol kerosene methods to a given community are analysed in terms of house room and population units.

5. The lowered total morbidity and malaria incidence rates for European service personnel stationed in the areas sprayed are quoted as evidence of the results achieved by the combined control measures employed and the extent of the spray killing scheme's contribution thereto is discussed.

6. Less specific evidence of benefits accruing to the non-European population is considered, with special reference to infant malaria index trends. It is shown also that the keeping of comprehensive spray killing statistics conferred the indirect benefit of affording useful supplementary data to Catching Station records, a valuable pointer to the efficiency of associated oiling and drainage methods of control, and also evidence of the wide variations in mosquito density which can occur in consecutive rooms.

VI.—CONCLUSIONS

It will be evident from the foregoing account of benefits direct and indirect, believed to have resulted, that the exact evaluation of the effects of the large-scale spraying scheme described is very difficult. From all the material recorded it

seems however reasonable to deduce, that these effects were appreciable and that a spray killing scheme should form an integral part of any emergency malarial measures undertaken, more particularly where the emergency is of a type such as one was faced with when the measures described herein were framed.

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Discussion

Professor R M Gordon I know we shall all agree that we owe a debt of gratitude to Dr EDDEY for a very interesting paper We all regret that he was not present to read it himself but we were very fortunate in having Wing-Cdr HACKETT to read the paper for him, in particular as Wing-Cdr HACKETT was in the Gold Coast at the time, or at any rate before and during the work, and I have no doubt he will be able to assist us in some of the points that are sure to arise.

I have just said we are all agreed it was a very interesting paper but I think it was more than that it was a very important paper and I believe very helpful one. Only too often work of this character is undertaken against malaria and much labour expended on it, but when the results are announced whether they are good or whether they are bad, it is found difficult to place the cause of the success or the failure, because there are not sufficient data given to allow those who come afterwards to repeat the observations and to learn how to avoid the difficulties and the failures of their predecessors. That is not the case with Dr EDDEY s paper it is a very carefully considered piece of work, every detail of which appears to have been studied.

I open this discussion with considerable diffidence. I have had no experience of anopheline control in West Africa, but I have had no experience of spraying, since this came after my time. Spraying as a form of control is no novelty I do not know who began it, it probably originated before I

d overy of the transmission of malaria but its scientific application on a large scale is of comparatively recent introduction That its importance in this form was early appreciated is I think shown by the work of such a distinguished malariologist as Brigadier COVELL, who in 1941 wrote that he thought that the most important advance in the past 10 years in the control of malaria, was the spraying of pyrethrum extract in oil against adult anophelines in houses

I am very glad that to-night's paper dealt with the development of this comparatively new method of control in West Africa For one thing the problems of control in West Africa have been particularly important in this war For another although, as we shall see in a moment, the control of anophelines by spraying originated in Africa, so much work has been produced subsequently from India, that I think it is time some work from Africa should again make its appearance. I believe the first point that arises is whether spraying has proved its value whether it has come to stay or is one of those things that must be used quickly before it loses its efficacy One of the first reports I think was that of DE MEILLON (1936) from Natal. He was impressed by the fact that the local vectors were house haunting species A gambiae and A funestus and he undertook a spraying experiment He reported, to use his own words Anti adult work costs only about a third of the anti-larval and moreover it was more effective Stimulated by DE MEILLON's work, COVELL, MULLIGAN and AFRIDI in 1938 repeated these observations on a larger scale in India. Their report states that the results suggest that this method is likely to prove of great value in India especially in the case of isolated communities where anti larval measures are impracticable and where the vector species of anopheline rests in dwelling houses during the daytime These encouraging results were quickly followed up by the Rockefeller workers in India, RUSSELL and KNIPE, who in 1938 using pyrethrum extract in admittedly unsatisfactory sprayers, showed in their first report the following satisfactory results In the treated villages in November 1937 the spleen rate was 68 per cent. in November 1938 this dropped to 24 per cent As regards the parasite rate in treated villages in November 1937 it was 57 per cent. and in November 1938, it dropped to 12 per cent. In the untreated villages the figures remained at a constant high level. Following this there was a series of very important papers on the subject from the Rockefeller workers in India. I will only quote a few figures from their last report dealing with the sprayed villages. Taking the parasite rate in the first village it fell from 57 1 to nil in the second village from 15 to 1 3 in the third from 71 4 to 1·6 in the fourth from 40 5 to 14 1 in the fifth from 45 to 10 8, in the sixth from 61 1 to 22·6 and in the seventh from 42 5 to 9·6 Again the control villages remained unaffected and as a matter of fact, they all showed a rise In summing up they conclude There can be no doubt whatever that the decline in parasite rates following spraying villages was due to the control

measures and not to natural fluctuations in the malaria curve. VISWANATHAN in 1941 and 1942, working in Assam confirmed RUSSELL and KNIPE's finding, basing his opinion on the hospital admission rate, and the parasite index, adding the significant fact that the malaria index in infants less than 1 old in the sprayed area was nearly double that in the unsprayed area in July, only half of it in September and November and about three-fifths of it in December the figures being for the entire year. This is a very important point, for as workers from the Liverpool School of Tropical Medicine in Sierra Leone have pointed out, when dealing with a super saturated infective density the most valuable guide to the effects of mosquito destruction in the native community are newly born children, since in older persons a great reduction the density may still leave them saturated with infection. In connection with this, DE MEILLON repeated in Northern Rhodesia observations he had made in Natal but was somewhat disappointed with his results.   He writes   On a mine in Northern Rhodesia for example rigorous anti adult campaign reduced the number of mosquitoes per hut 0.01 in other words, one mosquito to every ten native huts, yet it to make no difference in the incidence of the disease.   This is comparable to the Sierra Leone figures just referred to where the density in a native village was 1.3 whereas in a town subject to anti-anopheline measures, it was only 0.03 In both these areas persons over 2 years of were, for practical purposes, saturated since there was a parasite rate of nearly 100 per cent. But in infants of 3 months and under 5 per cent. were infected in the sanitated areas, whereas at the same age 50 per cent. infected in the unsanitated areas   at 6 months 40 per cent. were infected in the sanitated area and more than 80 per cent. in the unsanitated area. I is possible DE MEILLON would not have been so disappointed with the results in Northern Rhodesia if he had examined the infant population as a guide.

I was very much interested to note what Dr EDDEY said with to cresol kerosene being so effective as a spray since pyrethrums are so of in short supply   but I was rather surprised that the cost is almost as as for pyrethrin. There was another point which impressed me Dr Ennis mentioned in the paper which Wing Cdr HACKETT has just read, that attitude of the African native towards the ridding of his home of mosquito by spraying was very helpful and there is no doubt that Dr EDDEY received great co-operation from the local inhabitants and it is interesting to note KNIPE and his colleagues in India recorded the same attitude amongst the local natives. This obtaining of native co-operation in all attempts at controlling the anopheline population is, I think, of the utmost importance, since it make all the difference to the success or failure of such a campaign.

One last point, but it is really two points. Dr EDDEY's account shown that on the Gold Coast there is really only one anopheline vector malaria—*Anopheles gambiae* Now it has been recorded by DAVEY and other

workers in West Africa that the vast majority of anophelines of this species found in the house at 7 o clock in the morning have left it by 7 o clock the next morning Further it has been shown by BLACKLOCK recently that one of their resting places is in the bushes outside dwelling places   This raises two questions, and I do not know whether Wing-Cdr HACKETT will be able to answer them The first is whether any particular hour was selected for spraying the houses, and secondly whether spraying was attempted outside the houses ? In conclusion, I can only repeat how much we have appreciated Dr EDDEY s paper and Wing-Cdr HACKETT's kindness in reading it.

Lt -Col S P James I should like to associate myself with Professor GORDON and Wing-Cdr HACKETT in congratulating Dr EDDEY sincerely on the scientific manner in which he has dealt with this important subject. To workers of my generation it is particularly gratifying to learn that an anti malarial measure which was recommended by the Malaria Commission of the League of Nations more than 20 years ago is now being used by malariologists of the present day I remember that when the measure was described and recommended in the Malaria Commission s second general report a frequent criticism was that, although it might perhaps be worth while to try it in Europe it would be quite impracticable to endeavour to apply it in the tropics Dr EDDEY s paper shows that that criticism was not sound

Dr EDDEY has described the application of the measure in tropical Africa but I think it is fair to say that India, which has always been foremost in arrangements for malaria research and control was the first tropical country to test it on a considerable scale and as a result, to confirm its great value * I was interested to read, in a recent paper on results obtained in some villages in Southern India, that the spraying was sometimes done by the householders themselves This was the final aim which the Malaria Commission described as being very desirable and I was therefore glad that Dr EDDEY seems also to have succeeded in obtaining the collaboration of the village inhabitants to the extent, in some cases of getting householders to do their own spraying

Of course, up to the present, pyrethrum has been the insecticide of choice, but it is to be expected that much better results will be got when supplies of the new synthetic insecticide D D T become available One great disadvantage of pyrethrum is that it is a repellent of mosquitoes so they quickly fly out of rooms which have been sprayed with it   D D T on the other hand does not repel mosquitoes at all. They readily alight on walls which have been sprayed with it and, after resting on the wall for a minute or two they drop dead. Another unique advantage of D D T is its persistent action Rooms sprayed with it remain toxic to mosquitoes for several weeks so that spraying

* According to COVELL (1943) no other measure for the control of malaria produces such dramatic and consistently good results." *Health Bull* No 11 Malaria Bureau No 3 Anti mosquito Measures Sixth edition

at frequent intervals is not required. This outstanding persistence efficient of D D T has recently raised the question whether it might be incorporated in whitewash, distemper and even in some kinds of paint.* Indeed, having regard to these and other advantages that are claimed for this synthetic new trend, including its proved larvicidal action, coupled with the finding that illiterate, uncultured villagers who comprise the vast bulk of the population of tropical countries welcome its use in their houses one is tempted to prophesy that the liquidation of malaria as the greatest scourge the world has ever known may be nearer than we have ever hoped.

Dr F C Collingwood I would be grateful if Wing Cdr HACKETT would inform us whether the Westinghouse insecticide disperser—the freon bomb—has recently been improved to incorporate D D T in its constituents in addition to the pyrethrum extract formerly used.

Air Marshal Sir Harold Whittingham said that there were two main points regarding house spraying as a means of controlling malaria the first is whether spraying is worth while and the second is what is the best form of spray. He thought that outstandingly the best method of spraying is the aerosol bomb originally introduced to disinfest aircraft it is simple to use and very efficacious.

In the evaluation of spray killing of mosquitoes in native houses as a contribution to malaria control Dr EDDEY gives as principal evidence its effect on the incidence of malaria among the European (Service) population in the area. Thus, he gives the incidence of malaria in two groups of European (Service) personnel, comparing the years 1942 and 1943, and shows that in Group 1 there was a 57·3 per cent. reduction in the incidence of malaria, and in Group 2 a 65·8 per cent. reduction whereas, the incidence in Service personnel throughout the Gold Coast as a whole showed only a 50 per cent. fall in 1943 as compared with 1942. From this he argues that the local measures, that is house spraying in the Takoradi area accounted for the greater fall in this area. Air Marshal WHITTINGHAM was not convinced on this point as, if the incidence of malaria among Service personnel in West Africa is taken as a whole for the years 1942 and 1943 it will be found that in Gambia the reduction in the incidence of malaria among R.A.F. personnel was 62 per cent. in Sierra Leone 57 per cent. and in Nigeria 47 per cent. That is, the improvement was equally great in Gambia and Sierra Leone as it was in the Gold Coast. In Gambia Sierra Leone and Nigeria this lowered incidence was not due to the spraying of native houses, though there may have been spraying of Service quarters. The improvement was mainly due to general anti malarial measures, such as personal protection by means of suitable clothing using mosquito nets, the taking of suppressive mepacrine and general drainage of camp areas. One important point not brought out in Table VIII of Dr EDDEY's paper is

fact that the Group A Service personnel lived in unscreened quarters and showed a 57 per cent. reduction in the incidence of malaria, whereas the Group B lived in screened quarters and showed a 65 8 per cent reduction in the incidence of malaria. These figures are strongly suggestive of the value of house screening as a means of controlling malaria

Professor D B BLACKLOCK I should like to say how sorry I am that Dr EDDEY is not here, we are indebted to Wing Cdr HACKETT for reading the paper In 1940 when I was in Sierra Leone the problem there was the excessive amount of malaria among merchant seamen Freetown harbour being a very important place for war time shipping The first step taken in October of that year was to institute the spraying of all launches and lighters and as far as possible, of the ships in the harbour The next step was to start spraying the houses in the village of Kissy where are situated the oil tanks from which many of the ships refuel. So far as the effects of spraying are concerned, we cannot be sure what proportion they contribute to the whole of the good results obtained because at that time we were using every possible means to reduce malaria. The figure obtained by GORDON and DAVEY in a survey in 1932 of anophelines per room in Kissy in the month of July was over twenty-four In July, 1941 after thrice weekly spraying and other measures had been in force, the figure per room had fallen to just over one On the other side of the estuary all the villages were sprayed first once a week, then twice and finally every day During June July and August, on several occasions catching in all houses in some of the smaller villages yielded no anophelines, although they had previously been fairly numerous. Kissy village itself had a very bad reputation both as regards the malaria incidence and the numbers of anophelines it was therefore all the more pleasant to observe how the situation was improving In the interval between my leaving in 1941 and returning at the beginning of this year the British Admiralty had built there a large new naval dockyard. Spraying is still going on, and will go on until control measures of a permanent kind are put into force

When I was in the Gold Coast I had the pleasure of spending a profitable day with Dr EDDEY in the Takoradi region. He showed me the work he was doing and told me about the enormous numbers of houses which were being sprayed and the results he was obtaining I was impressed by one interesting observation which Dr EDDEY made namely that he thought that in some of the villages in the area, where miles and miles of drains had been cut, there was actually an increase of anophelines in the houses

The first effect of cutting earth drains in an anopheline area is that each drain lowers the ground water on both sides for a considerable distance As the sub-surface water falls the water of the surface pools soaks down into the soil and makes its way into the drain innumerable surface breeding pools are thus eliminated. The next effect however is in the drain itself where

large numbers of seepages occur As we know anophelines will often breed in a very small amount of water I remember seeing in Freetown a recently cut launch slipway down to the sea. On the exposed earth surface of the cutting there was a mere film of water derived from small seepages. Immense numbers of *Anopheles gambiae* and *A melas* were breeding in this thin surface water-film This place was very small and was easily dealt with. But when you make hundreds of earth drains you have many serious problems, several of which have been mentioned by Dr EDDEY in this paper In addition to the seepage there is silting of sand with pool formation again cattle get into the drain dislodging the earth which then forms dams. They walk along the drain sometimes for hundreds of yards and every now and then try to scramble out each time they bring down more earth. People follow them and try to dig them out so that the whole drain bed is pitted with foot marks in which water lodges and in every one of these foot marks you may find anophelines breeding

There were these drains, then enormous lengths of them, which had to be dealt with. But the military situation in West Africa was altering in that West Africa was ceasing to be an important centre of military operations. As a result there was a withdrawal of skilled personnel for the supervision of these anti-malaria works. The drains required continual supervision to keep them in order otherwise they were going to constitute a great danger throughout the whole area. I reported to this effect to the Colonial Office and advised that experiments should at once be made with local materials to carry out the well-known methods of sub-surface drainage. This would give a permanent result which could be relied upon with just a little supervision

I cannot see how we can dispense with spraying for a long time yet Dr EDDEY's work is the most comprehensive account so far given. He has enumerated the methods he used and also worked out the cost per head of population for each. The figure of one penny per head per week may seem quite small, but there are 52 weeks in the year and with say five people in a house the total would be quite a large sum for a person such as an African labourer earning very little money Dr EDDEY has done a valuable service by adopting the aim of carrying out his work scientifically and recording it accurately

Dr W H KAUNTZE I should like to add to Professor BLACKLOCK's statement about drainage schemes in West Africa that we have much in mind the dangers he envisaged and are trying to make permanent the anti malarial measures which have been started during the period of military occupation in West Africa. I may say that before very long we hope to have available a full account of all the measures which have actually been put into force to deal with malaria in West Africa during the past 4 or 5 years, and are also formulating a scheme for permanent anti-malarial works, so that in future temporary measures will not be necessary

The President   Dr EDDEY says that the working life of each dispenser is 2 minutes   What exactly is meant, in this connection, by ' working life ?

Wing-Cdr Hackett   There is no doubt that natives appreciate house spraying and will often complain if their houses are missed.

House spraying is of rather too recent introduction to expect native householders to carry it out for themselves at present.   It may be hoped for in the future but there were sanitary measures of longer standing whose application by natives lagged behind.

I do not know if D D T has been put up in freon dispensers.

As to the relative importance of house spraying and house screening Dr EDDEY's conclusion is that the greatest reduction in sickness was due to house spraying   Native house spraying in the vicinity of R.A.F. stations elsewhere in West Africa was also commenced early in 1942.

Dr V B Wigglesworth   It must indeed be gratifying to Colonel JAMES that one of the suggestions of the League of Nations Commission should have proved so valuable when submitted to the test of practice   We are in Dr EDDEY's debt for the care with which he has outlined his methods and evaluated his results and their cost.   During the war we have become accustomed to leaving out of account the question of cost   but as soon as the newer methods of malaria control developed in war are applied to civil use, the question of cost will become paramount.   It is most interesting that Dr EDDEY should have found so little difference between the cost of using a first rate insecticide like the pyrethrum aerosol and the relatively inefficient kerosene-cresol mixture   One would like to ask whether any difficulty was encountered in limiting the exposure of the Westinghouse bomb to the prescribed periods.   This apparatus lends itself only too easily to gross waste of insecticide

As Colonel JAMES has pointed out, since this work was done D D T has been developed.   There is some risk of too much being claimed for this insecticide, and it is perhaps unfortunate that in speaking of it Colonel JAMES should momentarily have dropped his native caution and assumed a less responsible tone.   But there is no doubt that D D T is a powerful new weapon in our armament against malaria.   Its novel properties are its stability and its power to kill, without repelling such insects as rest for quite short periods upon surfaces that have been treated with it.   It is a common observation in West Africa to see scores of blood filled *A gambiae* on the walls at dawn and for these all to have disappeared a few hours later   The opportunity for a residual spray of D D T looks promising   The development of a technique for applying such a spray in a form that would be at once effective and acceptable to the people, and practicable for use under civil conditions is a problem which I hope Dr EDDEY may be encouraged to take in hand and evaluate with the same discrimination as he has displayed in the paper to which we have just listened.

Dr L G Eddey (in reply by air letter) With regard to points raised Professor GORDON excepting for the trial wherein the daily spraying from 7 to 8.30 a.m. was specially mentioned, see Section VII (4) II of my paper the work commenced regularly at 7 a.m. and continued throughout a work day totalling 6½ hours, exclusive of the 30 and 90 minute breaks allotted respectively for breakfast and a midday siesta. Exterior spraying was restricted to that required for barrier zone purposes, and normally involved only wall and door apertures, or in unceiled rooms any open spaces between wall and roof. In view of the very interesting observations quoted by Professor GORDON it is hoped to arrange shortly for trial sprayings of the types of resting place mentioned. the aim would be to ascertain to what extent present interior kills could be augmented and whether, having regard to difficulties of achieving a lethal spray concentration under external conditions there is sufficient justification for the regular inclusion of external shelter places in future spraying programmes.

In reply to the PRESIDENT'S enquiry by the term "working life" meant the total period of time for which the dispenser would continue to function if its content were to be exhausted in a single spraying. Employed intermittently on small units as in the trials described each dispenser proved capable of repeated usage until the sum total of all its exposures had amounted to approximately 12 minutes.

Dr WIGGLESWORTH raised the question as to the difficulty of fixed dispenser exposures to the prescribed periods. I am able to state that, after patient instruction our African personnel readily acquired the ability to effect rapid adjustment of the apparatus at the beginning and end of each dispenser period once disciplined into practising the counting formula described in Section VII (4) I they achieved quite accurate standards of routine dosage.

Air Marshal WHITTINGHAM's contribution to the discussion is of particular interest in that he was able to enlighten the meeting with data of a type not normally available, as to the place of the Gold Coast malaria control achievements in the West African picture generally In doing so I am not afraid be unwittingly introduced a very questionable comparison. The 45 per cent reduction ascribed to the Gold Coast is correctly quoted as applying to Service personnel in general. The reductions quoted for the other West African colonies are described as referring to a specific Service only namely the R.A.F As given these figures are not strictly comparable and the inference to be drawn therefrom can hardly be accepted. It may now be disclosed that, taking R.A.F personnel only in Takoradi, a reduction of 65·8 per cent in the malaria morbidity was obtained—a better figure than either of those quoted for R.A.F personnel in general in the other West African colonies. On the Air Marshal's own evidence, therefore, the Takoradi achievement in behalf of the R.A.F would appear to have been an exceptionally good one. At the same time I would add hat in my own view it is very much open to doubt whether inter-colonial comparisons in respect of such matters as achievements in malaria con-

...in be justified. Even in neighbouring areas the ramifications of the initial malaria problems faced may differ so widely and the development of the control situation may be so fraught with purely local difficulties in regard to rainfall ... ards of public co-operation and individual interpretation of prophylactic disciplinary measures, that the obtaining of a single per centum reduction in one area may entail an expenditure of effort and ingenuity out of all proportion to that required to produce a similar reduction elsewhere

Regarding Air Marshal WHITTINGHAM'S statement that the Group A personnel referred to in my Table VIII lived in unscreened quarters I have to suggest that he has been badly misinformed. Whilst Group A's principal and lag behind those of Group B in initiating this measure the screening of Group A's messes and canteens was in hand by December 1942 and a large proportion of the European quarters had been protected by the wet season of 1943. The main reasons for the smaller reduction in the malaria incidence of Group A arose, in my opinion, from the fact that, as was not the case in Group B the duties of many personnel in the A service not only required that they live in a multiplicity of small scattered and purely temporary camp sites placed towards the fringes of the control area, but that they undergo a greater degree of exposure outside quarters during the hours of darkness. It must in any case, be borne in mind that the value of screening measures is necessarily limited by the fact that they exert no influence over personnel during those leisure periods spent in and about native townships where he concentrations of both the vector anophelines and of infective native adults and children. It was precisely this type of gap which existed in our anti-malarial defences at Takoradi during 1942–43 and there can I think, be little doubt that spray-killing alone of the remedial measures then feasible was capable of producing immediate results in respect of this breach.

COMMUNICATIONS

PYRETHRUM AS A TSETSE FLY REPELLENT HUMAN EXPERIMENTS

BY

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AND

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The effectiveness of pyrethrum as a mosquito repellent is now well recognized. In view of its success against mosquitoes it appeared to be of interest to investigate its possible action in preventing tsetse flies from biting human beings under bush conditions.

While preliminary laboratory experiments with pyrethrum were being undertaken HORNBY and FRENCH[†] published their results on the action of a number of compounds in preventing the bites of tsetse flies. The test animals used were sheep ox and donkey The only substance found, under laboratory conditions to have any repellent action on tsetse flies was pyrethrum which was used in the form of a 2 per cent. emulsion of pyagra (a proprietary pyrethrum extract sold for household use against mosquitoes and flies) in a 0·2 per cent. emulsion of soft soap The emulsion was sprayed on the animals.

A small field experiment with six donkeys carried out in Tanganyika, gave promising but not conclusive results. The tsetse flies employed were *Glossina morsitans*

C

HUMAN EXPERIMENTS.

The present experiments were carried out with "Antu mosquito (Mark II" which consists of pyrethrum in a vanishing-cream base.

The mosquito cream was kindly supplied to us by Colonel A. E. Reid A.S.L.S to whom our thanks are due.

Preliminary experiments, carried out in the shade of the laboratory results which pointed out the importance of further field experiments. I preliminary experiments the human left arm was well rubbed with the pr tion and the tsetse flies, placed in cages of gauze, were allowed to bite if wished at intervals. The results showed that tsetses failed to bite on the n skin though they bit on the untreated arm.

Field experiments were undertaken in a tsetse fly area in the Gold Co.

The place selected for the experiment was a wooded patch beside a r 4 miles on the road from Kintampo to Bambot, not far from the Fuller The tsetse flies in this area are predominantly *Glossina palpalis*. The wi was moderately heavy harmattan

Before each experiment four Africans were stripped to the waist and only shorts. Two Africans were anointed with antu mosquito cream on arms, trunk and neck two were left unanointed as controls.

During the experiment the participants sat beside the stream on fale trunks where there was dappled shade and light they changed places each other every 15 minutes to avoid one being in a place more suitable tsetse bites than the others.

They were instructed to keep perfectly still when they felt a tsetse and to say as soon as it bit.

At the end of each experiment those anointed were scrubbed with and water so that they could be used as controls on the following day a any one was naturally more attractive to tsetse flies than the others.

PROTOCOLS.
Experiment 1

10.30 hours   Cream applied
10 30–17 30 hours Exposed to biting *i.e.*, during the first 2 hours after applic

Controls.	Number of flies that settled.	Number of bites.
African A.	7	
African B.	3	4
Anointed		
African C	7	Nil
African D	6	Nd

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* One crawled up his shoes and bit him on the thigh where cream had not applied.

A fly was taken to have settled if it remained on the body for 30 seconds, or if i

Experiment 2.

14 15 hours   Cream applied.
14 15–15 45 hours   Exposed to biting during the first 1½ hours after application.

Controls.	Number of flies that settled	Number of bites.
African C.	5	5
African D.	12	12
Anointed.		
African A.	1	Nil
African B.	2	Nil

Experiment 3

09 45 hours   Cream applied.
11 45–12 45 hours   Exposed to bites during the 3rd hour after application.

Controls.	Number of flies that settled.	Number of bites
African A.	6	5
African B	Nil	Nil
Anointed		
African C	5	Nil
African D	5	Nil

Experiment 4

09 00 hours   Cream applied.
15.00–16 00 hours   Exposed to biting, i.e. 6 hours after application.

Controls.	Number of flies that settled	Number of bites
African C	6	6
African D	6	6
Anointed.		
African A.	6	Nil
African B	9	1*

Experiment 5

10.00 hours   Cream applied.
13.30–14 00 hours   Walked until sweating slightly
14 00–15 00 hours   Exposed to biting during the 5th hour after application.

Controls.	Number of flies that settled	Number of bites
African A.	3	3
African B	3	3
Anointed.		
African C.	5	Nil
African D	5	Nil

The Africans used in the first five experiments were not physically fit to take strenuous exercises so four strong healthy men were selected for the final experiment.

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* This bite was below the ankle, almost on the sole of the foot, from which all the anti-mosquito cream had probably been rubbed off by walking about during the 6 hours that had elapsed between the application of the cream and exposure to tsetse bites

Experiment 6

In this experiment all the four men wore boots, puttees and shorts.

08.30 hours	Cream applied.
10.50–11.30 hours	Chopped down trees with matchets in the sun, until sweat was freely pouring off them.
11.30–13.30 hours	Exposed to tsetse bites during the 4th and 5th hours after application.

All the men were immediately bitten.

Controls.	Number of flies that settled.	Number of bites.
Tumbu Wala	11	11
Sana Dagarti	7	7
Anointed		
Awurd Frafra	9	9
Lantu Dagomba	5	5

Total figures for Experiments 1 to 5 where no strenuous exercise was taken, are as follows —

	Number of flies that settled.	Number of bites.
Controls	47	46
Anointed	51	1

The experiments indicate that anti mosquito cream Mark II provides protection against bites from tsetse flies up to at least 6 hours after application provided that by work in the sun severe sweating is not provoked. The cream does *not* discourage tsetse flies from settling on the skin.

Exposure to sunlight rather than sweating may be the factor that nullifies the repellent action of the pyrethrum cream.

It was suggested that the cream might be useful for hygiene personnel working near heavily infested streams—for workers building bridges and for the boys   but that in its present form it would be very little use for troops on manoeuvres if the skin were exposed to prolonged direct sunlight.

As tsetse flies are less numerous during the harmattan, the experiments are to be repeated during the wet season when tsetse flies are plentiful.

Conclusion

Anti mosquito creams Mark II containing pyrethrum in a vanishing cream base, proved of value as a tsetse fly repellent up to at least 6 hours after application.

The repellent action was destroyed by heavy sweating associated with exposure to a strong sun.

ADDENDUM

Since the above experiments were made it has been possible to carry out two additional tests which tend to show that the action of the pyrethrum ointment is interfered with, at any rate in the case of man more by sweating than by the action of the sun. The technique was similar to that employed in the former experiments, African fly boys being used. They were clad only in shorts. The experiments were carried out at Yapei on the White Volta during the 1st week of April, 1944 the sky was clear and no rain had fallen for the previous fortnight.

Experiment 1

Three " fly boys " were rubbed with ointment except on the thighs under the shorts One untreated " fly boy " acted as control. Two treated ' fly boys danced for 30 minutes inside a native hut they sweated heavily another treated boy sat in the sun for 30 minutes The four boys then adjourned to the edge of the riverine bush where they sat in the shade changing places at intervals for a period of 1 hour

The following results were obtained :—

| | Number of tsetse | | Previous treatment. |
	Settling	Biting	
Adama	7	6	Sweating
Lawi	9	5	"
George	12	1*	Sat in sun
Amadu	15	15	Control.

Experiment 2.

One African " fly boy " after being rubbed, cut bush with a matchet in the shade for 30 minutes three sat in the sun for the same time after being anointed, while a fifth was untreated and acted as control.

| | Number of tsetse | | Previous treatment. |
	Settling.	Biting	
Kwaku	5	2	Sat in sun.
Kofi	4	0	"
Isa	6	0	"
Mansa	6	4	Sweating
George	9	6	Control

The two experiments may be summarized as follows :—

Untreated controls	24 tsetse settled,	21 bit.
Sitting in the sun	22 "	3 bit.
Sweating	22 "	15 bit.

Thus in the case of man heavy sweating is more deleterious than the direct action of sunlight to the protective action of the pyrethrum ointment.

Incidentally it has been found that pyrethrum ointment gives a considerable degree of protection against the bites of *Culicoides sp* In the early part of the rains these insects are a pest in this portion of the West African rain

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* Three tsetses crawled up his shorts and bit him in the groin on untreated skin.

forest. After smearing the whole body only two bites were received in the a control sitting alongside was bitten forty two times.

An experiment with *Hippobosca* sp in cattle showed that this fly will remaining on any area anointed with the ointment. A young bull, acting host to hundreds of hippoboscas, was smeared with pyrethrum ointment two of the most favoured areas, on the left shoulder and on the under sid of the abdomen. During the next hour only ten flies settled on the treated areas when they alighted they promptly flew away again or proceeded crawl to the edge of the treated area till they reached untreated skin.

ROUGH NOTES ANOPHELES MOSQUITOES AND MALARIA IN ARABIA

BY

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It is perhaps true to say that we are more ignorant of the principal diseases of Arabia than of any other large area in the Old World. It has seemed worth while to set down the little that is known about malaria as a starting point for others the matter may have an increasing urgency if some of the natural resources are to be developed in the near future.

The present notes refer to all the Arab lands in Asia, excluding Sinai Palestine, Transjordan, Syria and Iraq Very little is known from the area under review and most of what is recorded is based on the random observations of travellers it is notable that nearly every traveller from DOUGHTY to PHILBY or SCOTT refers to the prevalence of the disease in at least a part of the area he has visited. In his general account of medical conditions in Arabia, STORM (a medical man who has travelled and lived in several parts of this country) puts malaria at the head of the list at least so far as the coasts are concerned. All medical work on the coast has malaria for its background reinfection is continuous and certain (STORM 1938)

It would be impossible to present a connected account of the subject the data are therefore set down in the form of notes Where possible spleen rates are quoted but they must be accepted with some reserve owing to the presence of considerable amounts of urinary and rectal schistosomiasis at least in South West Arabia (PETRIE and SEAL, 1943)

MALARIA IN WESTERN ARABIA.

This area includes the Red Sea coastal belt and hills behind, to the main watershed. The coastal belt (Tihama) is much of it dry and sandy But where there is water malaria occurs very seriously though only in certain small,

well defined areas. Thus it appears to be absent from Akaba (children's spleen rate stated to be nil in 1937 by Department of Health, Transjordan. There is no information from Yanbo. At Jedda, malaria is evidently quite serious problem. Cases diagnosed on clinical evidence as malaria" found 45 per cent. of all out patients at the Government Hospital in 1935 and the British Legation dispensary reported a rate of 25 to 40 per cent. (in 404 visits) in the different years, 1931–1933. The spleen rate in 411 boys was 8 per cent. (MACKIE 1937) It is an interesting point that malaria should be so abundant in a place where water is so scarce it is thought that the anopheles breed in shallow brackish wells, and possibly in subterranean cisterns

Owing to the presence of *Anopheles gambiae* the southern part of the coast belt is probably very malarious, where it is not arid. There are Italian reports of grave malaria at Luheiya (Loheja), Hodeida, Mokha. Farsan and the other islands in the Red Sea are nearly waterless and probably free of malaria.

In the hills, it seems extremely probable that all the fertile lower valleys which run westwards from the mountains of the Hejaz, Asir and the Yemen are malarious, though few precise records are available, e.g. Nefr el Abid on the road from Hodeida to Sana has a bad repute, partly because it is a place at which travellers frequently pass the night. Moreover *A gambiae* is recorded from Jedda and Wadi Liya (a little north-east of Jizan), and is probably widely distributed, which would account for the malaria. There is said to be much malaria in Mecca and Taif (5 500 feet) but little or none at Medina.

The upper limit of malaria in the hills has not been defined it is said to be absent from Sana 8 000 feet (PETRIE, 1939). Taking into consideration work which has been done in Eritrea (under rather similar geographical and entomological conditions), it seems that native malaria is not very rare in this country at 6 300 feet and can occur at 6,600 feet (nz BURCA and IMPAS SHAH 1943) There is a record of locally acquired malaria (both benign and malignant tertian) at Addis Ababa, Abyssinia, 8,000 feet (MARTIN 1942).

MALARIA IN SOUTHERN ARABIA

The peninsula of Aden, on which there is a considerable European quarter and a large Arab town, is unlikely to provide many breeding places for anopheles it is very porous, and much of it very steep the rainfall is very low and the inhabited parts appear to be under careful sanitary control Consistently with these facts, malaria acquired on the peninsula seems to be quite rare, e g., in 1938 the Government Hospital treated 1,292 patients, diagnosed as malaria, all but eleven of whom came from areas outside the colony Occasionally however malaria occurs under circumstances which show that it was undoubtedly acquired on the peninsula. There was a remarkable small outbreak at the end of 1938 ten Europeans (R.A.F.) being admitted to hospital

or malignant tertian malaria within less than 3 weeks   all lived in barracks
or married quarters, and none had spent a night outside the Settlement area
since arrival in Aden   On this occasion in spite of careful search, no local
breeding was discovered.   Other similar cases acquired in the peninsula or
in Khormaksar, have occurred more recently   PHIPSON (1934) after long
experience, has also called attention to the occurrence of locally acquired
malaria, as a rarity, in residents in European quarters at Steamer Point   He
attributed the infection to anopheles being blown across the harbour from
Hiswa   the distance is no more than 4 miles.

The following figures give the incidence (per 1 000 per annum) in
Europeans in the Royal Air Force stationed at Aden —

	R A F		R A F
1933	3 2	1938	20·9
1934	3 2	1939	21 2
1935	1 3	1940	37 8
1936	2·4	1941	5 5
1937	4 7		

Even in peace time it may be difficult to know to what extent the disease
had been acquired in the immediate neighbourhood of Aden.   The figures
for the war period doubtless include at least some infections acquired on service
elsewhere.

The low ground (Sheikh Othman, Lahej Hiswa, etc) corresponds to
the tihama of the Red Sea littoral.   It is mostly desert, but some of the
oases are large and populous   They are watered by springs and some of the
valleys (e g the Wadi Kabir) hold water after rain has fallen in the hills   much
of the population lives close to water so that malaria is frequently grave though
very localized.   Sheikh Othman is a fertile oasis and small town some 10 miles
north of Aden.   It was intensely malarious   many European members of the
staff of the Keith Falconer Mission Hospital died of the disease in the 80s
and '90s   up till 1931 the hospital treated 200 to 600 cases per annum, but
by 1933 there were only eleven cases all of whom had resided elsewhere   The
successful reduction in malaria followed some very simple and inexpensive
control.

The north side of Aden harbour (Hiswa, Bir Rubak, etc) is liable to
flooding when the Wadi Kabir brings much water from the hills especially
in August and September   the amount of mosquito breeding is increased
because cultivators block the stream in order to irrigate, and in borrow pits
water may remain for months   *Anopheles* larvae, mainly *A culicifacies* are
abundant.   In spite of this no enlarged spleens were found in forty five children
in Hiswa, in 1938 (R A F unpublished data)   The recent work of PETRIE and
SEAL (1943) gives useful spleen rates (children, 2 to 10 years) for a number
of low lying places in the Protectorate   The rates range from nil at Shuqra

and El Waht (in both of which *A gambiae* was collected) to Zinjibar (68 per cent.) Lahej (84 per cent.).

It is generally thought that malaria in the low land north of Aden is mainly due to *A culicifacies* var *adenensis* which is certainly common. The mosquito control at Sheikh Othman was aimed at this species which was breeding in fresh water wells in gardens and potteries, and also in irrigation channels some of them very saline. To what extent *A gambiae* is present near Aden (though apparently overlooked) is not certain. It is widely distributed in the Protectorate

From the hills of southern Arabia some precise records are available. PETRIE and SEAL (1943) give the following children a spleen rates —

Place.	Spleen rate.	Place.	Spleen rate.
Tor am Baha	79	Al Milah (in Wadi Milah)	77
Dar am Fasha	25	Dhubiyat	*¶
Musemmr	85	Jebel Jehaf	80

These figures help to define the upper limit, in altitude of the distribution of malaria. The altitude of the villages on Jebel Jehaf (the summit of which is 7,800 feet) is unknown   it seems evident that in those villages there is no locally acquired malaria   for seven of sixty-eight children aged 2 to 10 had splenomegaly   It seems that Europeans have been infected while living at Dhala (5 000 feet) and *A gambiae* has been collected in the Western Aden Protectorate in many places including Dhala. On rather general grounds SCOTT (1942) believes that malaria is particularly prevalent at Al Munah (Upper Wadi Tiban, 3 800 feet) and at Musemmr in the Haushabi country   It is evident that the disease is serious and widespread in the Western Aden Protectorate and the Yemen, an important matter for the population of the hills is relatively dense.

Some parts of the Hadhramaut valley are probably malarious   there are certainly places in the upper part of the valley west of Shibam where dates are grown by people who visit them only for the harvest because of the malaria risk (PHILBY 1939). The R.A F has had men infected either at Makalla or in the Hadhramaut valley On the other hand, INGRAMS (1936) with good knowledge of the country implies that malaria is not in general, important.

MALARIA IN CENTRAL ARABIA.

Many travellers have spoken of oasis fever   There is little doubt that this disease is (for the most part) malaria, for DOUGHTY refers to the enlarged spleen the ague cake   moreover outbreaks following floods have been observed, as at Khurma. The diagnosis of malaria was confirmed at least for a part of the Hejaz, by MACKIE. Oasis fever was long ago recorded as grave at Kheibar (DOUGHTY). At Qasar 10 miles from Hail, a colony of settlers was wiped out and the area abandoned except for a few negroes (PHILBY)

erbat) Salwa also has been deserted owing to fever (PHILBY   Empty Quarter '), and other recent agricultural colonies *e g* Jabrin have suffered the same fate   There are records of the disease apparently less serious at several other places.   The occurrence of malaria in isolated spots in a vast desert is precisely similar to what has been observed in remote oases in the Algerian and Libyan Sahara, and in the Western Desert of Egypt.   The cause of the disease is that water is so scarce that the population (settled and nomadic) must crowd together where it and the mosquitoes occur

Malaria is said to be uncommon in Hail Riyadh, and most of Nejd.   There seems to be no record from Wadi Sirhan and the Jauf

MALARIA ON THE NORTH-EAST COAST

There are detailed reports from Muscat and Bahrein and clinical notes from a hospital on the coast of Al Hasa.

Bahrein is important because of its oil wells.   A full malaria survey was carried out in January and June in 1938 by the Malaria Survey of India (AFRIDI and ABDUL MAJID 1938)   It is evident that malaria is a very common disease, for the fever cases " constitute 20 to 30 per cent. of all hospital cases surgical and medical   they are most frequent in the period April to June and again in October to November   Moreover spleen rates are rather high   39 per cent. in 234 boys in the town of Manama, 13 per cent. in 137 in the town of Muharrak, and similar rates in several other spots   The parasite rate in winter was 14 per cent. among 249 boys drawn from all over the area   In this sample *Plasmodium falciparum* was found ten times *P vivax* thirteen times, and *P malariae* ten times   it is probable that the relative incidence of *P malariae* the parasite of quartan malaria, would have been lower in the hotter parts of the year   Summing the matter up there is good evidence that malaria is exceedingly common

The abundance of malaria in Bahrein is easy to understand for there are many types of fresh water on the island and the groves of date palms cover some 20 square miles   Some of the dates are irrigated by gravity an abundant supply of slightly brackish water issuing from copious springs, but there are also large open wells from which the water is raised for irrigation, and also artesian wells in a part of the area.   Moreover water is conducted from water-bearing strata near the hills through underground channels known as *falaj* to reach the surface on lower ground.   Much of the ground is water-logged the subsoil water is near the surface and seepages are very numerous.

Several species of *Anopheles* are very common, the predominant one *A stephensi*   It breeds chiefly in agricultural drains and leakages from them also in shallow domestic wells   indeed, larvae were found in 25 per cent. of seventy five wells in the town of Manama   In this respect the habits of this insect are much as they are in the Basra area   it appears not to breed in cisterns

or roofs as it does in Bombay The adult is frequent in houses, and appears to be able to travel at least 1½ miles. It is the only species in which infections they only found eight mid-guts and one gland infected. Three other species *A fluviatilis culicifacies* and *sergenti* none of which appears common, might perhaps be associated with the transmission of malaria to *A culicifacies*, it is remarkable that that has not been found breeding in wells, its habit in Muscat and Aden. *A pulcherrimus* breeding abundant in swamps and stagnant grassy drains, is not likely to be associated with transmission of malaria.

Afridi and Abdul Majid made detailed recommendations for the control of *Anopheles* and malaria in Bahrein. Among other things they emphasize the importance of vertical drainage of the water logged areas and of the use of a local larvivorous fish. It seems clear that the use of oil has much to recommend it for it must be readily available owing to the local oil wells and refineries.

Further information has been made available by the Bahrein Petroleum Company Their typescript reports striking evidence of the value of screening which is combined with air conditioning The total American and European personnel numbers about 500 in 1938 before the installation of screening they lost 860 man-days through malaria (not a very high figure) In the following year the loss was 60 days, though there is still a considerable amount of malaria among members of the staff who carry out their duties at night. It is noted that among the native population benign tertian malaria is much commoner than malignant, but the diagnosis is perhaps clinical. *A stephensi* is commoner than any other species among adult mosquitoes captured in houses Of those dissected 0 5 per cent. were infected with *Plasmodium*, the rate rising to just over 2 per cent. at the transmission season. Number of dissections not stated.

The Californian Arabian Standard Oil Company maintains a hospital at Dhahran in the Hasa, on the Arabian coast. The medical officer reports that in 1941 among 2,133 cases of "contagious diseases" he saw 373 cases of malaria, i.e. 17 5 per cent. It is not possible to calculate the proportion of malaria to total out patients. Among this series of cases, malaria was commoner in the second half of the year (2½ per cent.) than in the first (8 4 per cent.) A proportion of the cases were more thoroughly examined and the parasite of malignant tertian malaria (*P falciparum*) was found in 19 the benign tertian malaria (*P vivax*) in 20 *P malariae* was not seen. These observations are made mainly on the company's employees, some of whom are recruited from regions as remote as Nejd and the Hejaz. The general impression is that these two forms of malaria are both common along the coast of Al Hasa and that malaria is widely distributed inland.

For a knowledge of malaria in Muscat we are indebted to Gill, who served here for rather over a year as medical officer in charge of troops, and

than a quarter of a century ago   The disturbed state of the country prevented him going outside the immediate neighbourhood of the town (GILL, 1916) GILL found that ' fever ' was exceedingly common throughout the year among troops the rate of admission to hospital for fever ranged between 200 to 400 per thousand per month in the colder part of the year   in the hotter months the figure was generally rather under 100   A similar seasonal difference was apparently observed among civil cases   There was some evidence that malignant tertian malaria was commoner than benign tertian   quartan was seen, and a few cases of blackwater fever were recorded.   GILL found that *A culicifacies* was common in the colder months breeding in a large borrow pit and occasionally in wells and masonry cisterns   it seemed to be much rarer in the hot weather   *A stephensi* appears to have been rare and three other species were taken.   GILL is probably correct in thinking that *A culicifacies* is the most important carrier of malaria.

There are a number of general statements in HARRISON s recent book (1940)   it is evident that malaria is very common serious and widespread in nearly all parts of Oman   there is a very large population of cultivators in oases   indeed Arab estimates put the oasis population at 200 000 of whom half live in the Botuna (which is presumably a fertile depression)

ANOPHELINE FAUNA.

It is believed that the following is a complete list of species recorded from Arabia.

Anopheles coustani (mauritianus)   A single worn specimen (apparently var *enebrosus*) from Dhufar, S E coast, Long 54° (B S THOMAS   see EVANS 1938 p 73)   No other Arabian record

A d'thali (not *rhodesiensis*)   Muscat (GILL)   Ta iz Yemen (Dr TOF OLON in London School of Hygiene and Tropical Medicine)   Aden also Wadis Tiban Maidin Huweimi and other localities in Western Aden Pro ectorate.   (Dr P W R PETRIE, specimens in London School of Hygiene and Tropical Medicine   pharynx not examined but specimens show all the external characters of *d'thali* as indeed is to be expected from this area)

A sergenti Bahrein (AFRIDI and ABDUL MAJID)   This appears to be the only record from the vast area between Egypt, Syria and Palestine on the west, and Waziristan.

A culicifacies   Aden hinterland (CHRISTOPHERS and KHAZAN CHAND   HIPSON)   Muscat (GILL)   Bahrein (AFRIDI and ABDUL MAJID   Hodeida Dr MERUCCI in London School of Hygiene and Tropical Medicine)   The material from Aden Hinterland was identified by CHRISTOPHERS and KHAZAN CHAND (1915) as *A culicifacies*   Later it was distinguished as var *adenensis* (CHRISTOPHERS 1924) which differs from the Indian form in that the pale reas on the costa are much broader   still later (1933) CHRISTOPHERS figured differences between variety and type in phallosome leaflets.   The material from

Hodeida (of which we have males and females) is var *adenensis*, which has recently been recorded from Assab in S. Eritrea (DE BURCA and SHIL,). On the other hand, as AFRIDI and ABDUL MAJID do not refer the specimens from Bahrein to the variety one assumes that they are typical. In view of differences in appearance and genitalia, and of the fact that near *Adenensis* insect breeds in wells (an unusual habit in Indian *culicifacies*) it may be that *adenensis* is a distinct species. It certainly requires further study

A. arabica Muscat (GILL). The status of this is obscure pending careful collecting CHRISTOPHERS and PERI (1931) examined a larval skin concluded that it is not *funestus* but probably near *fluviatilis*

A. subpictus Stated to occur in Aden (PUTISON 1934). The record requires confirmation there is no other record of the occurrence of this species west of India.

A. gambiae (arabiensis PATTON). Jedda (1936, MACKIE, British Museum Wadi Liva N. E. of Jizan, Red Sea Coast, 43 E. 17° N (1937 H. St. J. Philby, British Museum)   TAIZ, Yemen (TOFFOLON)   Aden Hinterland (PATTON, also CHRISTOPHERS and KHAZAN CHAND)   Aden, also Wadi Maidan, Dhala (ABYAN) Shuqra, Lahej and other places in West Aden Protectorate (Specimens in London School of Hygiene and Tropical Medicine)   PATTON's map PETRIE's specimens, and records in the *Aden Protectorate Medical Service* show a wide distribution in the West Aden Protectorate, along rivers and up to Dhala (5 000 feet).

A. turkhudi (a-riki PATTON). Madruga, 134 km. from Jedda, about 40 lat. 22° 2,000 feet (MACKIE, in British Museum)   Arrik and other localities Aden Hinterland (PATTON)   Hiswa, near Aden (R.A.F. unpublished)

A. cinereus (jebafi PATTON)   Two specimens in British Museum, label *cinereus* var ? collected in Aden Hinterland (PATTON)   the must have been examined by EDWARDS. The old record from Muscat (GILL) could hardly stand by itself in view of the close resemblance of this species to *turkhudi*

A. multicolor   Twelve specimens in British Museum labelled Jeddah 1936 F P MACKIE" The material must have been seen by EDWARDS and the identification is doubtless correct. The locality was apparently open to some doubt.

A. stephensi Bahrein (AFRIDI) Muscat (GILL) Steps should be taken to discover whether *A. stephensi* in the Persian Gulf and Lower Iraq corresponds to one or other of the races which have been distinguished in India

A. theobaldi Male and female in British Museum labelled " Arabia, Aden Hinterland, Capt. PATTON As there is no other record from countries West of India and as the species is not among those listed by PATTON himself it seems probable that an error in labelling has occurred.

A. pulcherrimus Bahrein (Afridi).

A. pretoriensis (tibamus Aden Hinterland, PATTON). Wadi Maidan (PATTON)

My colleague, H. S. LEESON, who cannot at present be consulted, examined larvae collected by PETRIE from Wadi Natid Western Aden Protectorate they did not appear to represent any of the above species but seemed to have the characters of *macmahoni* and *rupicolus* Further material is required

The above list includes fifteen species (excluding two doubtful larval determinations) and two others (*subpictus* and *theobaldi*) should be omitted. The occurrence of the remainder is well established though some of the older locality records, made with all due care may require reconsideration in the light of recent work in other countries In any case of doubt it is desirable to collect a series of adults of both sexes, and also larval and pupal skins. Points which might cause difficulty in the field, or with imperfect material are the separation of *sergenti culicifacies fluviatilis* and *arabica* also of *turkhudi multicolor* and *cinereus* (and also *hispanola* now shown to be common in South-West Transjordan, close to the borders of Saudi Arabia and therefore likely to occur in the area studied in this paper LUMSDEN 1944) The status of the variety *adenensis* of *culicifacies* requires further work.

It is evident that there are immense gaps in our knowledge of the distribution of species indeed no species has ever been collected from the interior of Arabia. Further work may also add to the list for instance, A *hyrcanus hispanola* or even *superpictus* (known from Sinai) might occur in the north and A *funestus* or some other unrecorded African species in the south west.

MALARIA VECTORS

In the absence of dissection (except at Bahrein) one must rely on work done in surrounding countries in attempting to suggest which species are actual transmitters of malaria. It seems probable that the principal vectors are —

(a) Western Arabia. A *gambiae* further definition of its range is a matter of great practical importance

(b) Aden and Southern Arabia A *culicifacies* is generally regarded as the transmitter in and about Aden. In this area it breeds in fresh or salt water in open agricultural wells and in channels in gardens. The importance of A *gambiae* commonly breeding in pools in stream beds, has not been sufficiently realized.

(c) Central Arabia There is no information. It is possible that, as in the Libyan and Algerian Sahara, A *sergenti* may yet be found breeding in fresh water in oases and A *multicolor* in salt waters either might probably transmit malaria.

(d) North East Arabia. A *stephensi* has been found infected at Bahrein and is doubtless an important carrier as it is in lower Iraq A *fluviatilis* *sergenti* and *culicifacies* might also transmit. In Muscat, GILL was inclined to regard A *culicifacies* as the probable vector but he also collected *stephensi*

CONTROL OF ANOPHELES AND MALARIA

It is not appropriate to give an account of technical methods for the detection of mosquitoes and control of malaria. Attention is directed to the fact that many of the malarious areas are very small. It might therefore be possible not merely to reduce but to exterminate *Anopheles* from some of the more isolated oases and wadis once that was done it is unlikely that the mosquito would recolonize the area, owing to the enormous extent of waterless country. But this cannot be done till proper surveys for malaria and *Anopheles* have been carried out

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OBSERVATIONS ON *ANOPHELES GAMBIAE* AND OTHER MOSQUITOES AT WADI HALFA

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INTRODUCTION

Wadi Halfa lies a short distance south of the boundary between Egypt and the Anglo-Egyptian Sudan. It is an important town on the Nile route because it has an aerodrome and is a terminus for railway and steamer services. *Anopheles gambiae* has recently been found in central Egypt, and very thorough measures of control of this species are necessary at Wadi Halfa to prevent it from either spreading northward and adding to the problem of control in areas where it now exists or invading new areas further north. If this mosquito can be exterminated in Egypt, Wadi Halfa may then serve as a barrier confining it to the south.

It seems desirable to record what we know of *A gambiae* and other mosquitoes in the area and the conditions which affect them. A reason for considering several aspects of the subject at some length is that the Aswan

D

Reservoir may be raised above its present height (NEWHOUSE, 1929). It would probably extend swampy conditions which now exist only on a small scale

The writer visited Wadi Halfa in June, July August and October, 1942 and in February April May and December 1943. At other times information was supplied by the Public Health Staff or by an entomological assistant.

DESCRIPTION OF THE AREA.

Wadi Halfa, the most northerly town in the Sudan, is situated near the southern end of the navigable reach of the Nile between the Second Cataract and Aswan The area with which we are concerned extends from the southern end of the Cataract to Faras some 58 km. downstream on the frontier, and for a short distance on each side of the river (see Map below).
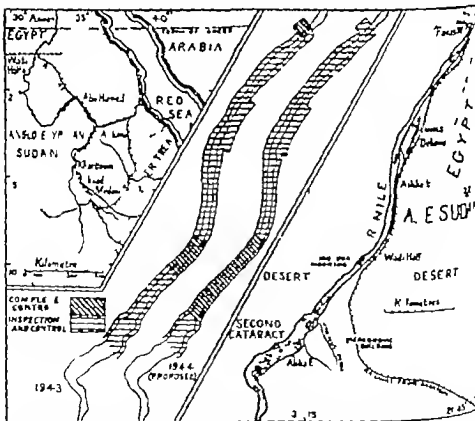
Map —The Wadi Halfa area with diagrams showing the anti-larval "barrier" designed to prevent the northward spread of *A. gambiae* from the Second Cataract Inset: part of the Nile Valley

The river —In the Second Cataract, which is about 13 km long, the river
flows rapidly among islands. HUME (LYONS, 1906) described it as follows
        we find numbers of islands, some sixty being fairly large, while the
total number reaches about 200   the principal rocks are a series of dark
hornblendic rocks, which are often much crushed, and are cut by numerous
dykes of dolerite and other rocks, enabling the river to erode a network of
channels most of which are shallow and dry at low stage." Several of the
channels contain pools and sheltered inlets, some measuring hundreds of square
metres and containing patches of *Potamogeton crispus* L   *Naias* sp and fila-
mentous algae.

Below the Cataract the Nile is nearly straight and flows between steep
banks of alluvium. There are several large islands and sand banks some of
which change their position in the course of a few years   Numerous pools
are formed on them at low water   In a few places particularly near Wadi
Halfa and Ashkeit, there are sedge-covered mud banks along the shore which
are exposed at low water   They are pitted with numerous depressions a square
metre or more in area which form pools (Fig 1)   A sample from one such
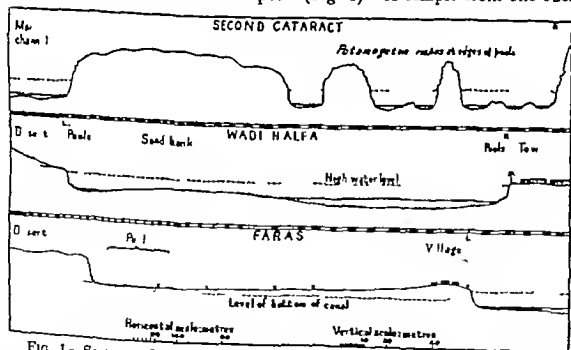
FIG. 1 —Sections of the Nile at the Second Cataract, Wadi Halfa and Faras (left
bank) looking downstream. Dimensions are approximate.

bank was found to comprise coarse sand (8 per cent.) fine sand (20 per cent.)
silt (11 per cent.) and clay (62 per cent.)   In spite of the binding effect of the
sedge roots, these banks alter in size to some extent. Some pools are formed
in the channels of water wheels as the river falls, others in holes dug for water
in sand banks and other by water seeping out of the banks. An unusually
high river pours water over its banks in a few places, producing small temporary
swamps sometimes 50 metres or more inland.

The annual variation in river level is about 6 5 metres with a maximum in September and minimum in June (Fig 2). Wadi Halfa is on the upper part of the Aswan Reservoir which causes the water level to remain for some months about 3 metres below the flood level, this difference naturally being greater at the Second Cataract and less at Faras. The natural discharge of the Nile at Aswan is above Egypt s requirements from August to January but October the amount of silt in suspension becomes small enough to permit storage without risk of silting. The effect of storage usually reaches Wadi Halfa early enough to prevent much pool formation in November. The level is held with minor fluctuations till May when it falls rapidly
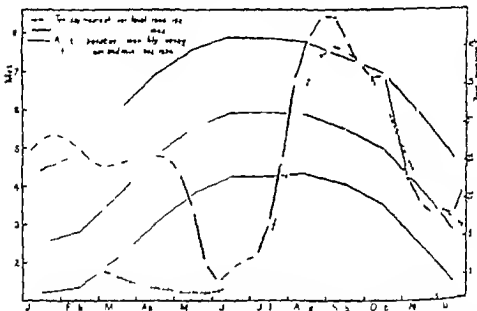
FIG 2.—River levels at Wadi Halfa, before and after the second raising of the Aswan Dam in 1934 and monthly mean air temperatures. The zero of the river gauge is 114·05 metres above sea level.

Communications.—Trains from the south normally stop at Wadi Halfa but continue to Faras when a low river level hinders navigation. Steamers start from Wadi Halfa or Faras, bound for Egypt. Flying boats alight at Wadi Halfa and aeroplanes land some 12 km. to the south. Most sailing boats trade between Wadi Halfa and Egypt, only a few sailing south of Wadi Halfa. Very few come from the south pass through the Cataract. Most of those bringing the annual date crop unload at Abka. There is little motor traffic outside the town.

Riverside areas.—On each side of the river is the desert, in some places reaching the water s edge and in others separated from it by an old flood-plain

hundreds of metres in width. Much of this plain is irrigated by pumps (at Dueira and Wadi Halfa) and by *sakias* These are water-wheels which raise water from the river or from wide wells or *mataras*

At Faras West the low land between the river and the desert was to have been irrigated some years ago by the basin method and to this end an inlet and outlet canal were dug Unfortunately reservoir water seeped underground through a layer consisting partly of gravel The land became water-logged and salts rose to the surface, so that the scheme had to be abandoned Seepage water flowed partly along the canal and partly underground to form brackish pools at some distance from the river In the canal grew *Potamogeton nodosus* Cham. *P pectinatus* L., *Nitella* sp *Typha* sp *Phragmites* sp and a filamentous alga Water entered the canal in August and disappeared in July and the pools (their size partly controlled by pumping from the canal) existed from the winter till June Water taken from five pools on 13th April, 1943 was found to contain from 0·18 to 1 33 per cent. chlorine and from 0 26 to 0 45 per cent. sulphate, much of it in the form of salts of sodium The Faras basin is to be reclaimed but, in view of the possibility that other swamps may be formed in the future, the salt content of the water is of considerable interest in relation to the breeding of *Anopheles gambiae* In a large part of the Nile Valley river water enters the banks in the flood period and, when it flows out later brings from the soil salts in solution, thus increasing the salt concentration in the water of the river when the level is low For example seepage water collected from the left bank opposite Dueira before the present regime of the reservoir was found to contain 0·04 per cent. chlorine (WILLCOCKS and CRAIG 1913 p 62) At Faras the effect of the reservoir is to reduce the period when salts are extracted from the soil and also to add salt to the soil at a period when the concentration in the water of the river is near its maximum Some of this salt, together with that already in the soil is brought to the surface where the water rapidly evaporates so that, presumably seepage swamps of the Faras type tend to becomes steadily more salt.

Climate—Wadi Halfa is hot in summer and cold in winter Rain is almost unknown and the air is dry The wind is almost always northerly and, when it blows from the south, does so mainly by day Calms are frequent.

The following figures, except where otherwise stated, are taken from records made between 1902 and 1934 The mean maximum shade temperature in June is 41 4° C (106 5 F) and the mean minimum for January 7 8° C (46 1° F) The highest recorded maximum is 52 5° C (126° F) The lowest temperature ever recorded is — 2·0 C frost sometimes occurs, on not more than two days, in December Monthly mean temperatures, calculated as (8h. + 14h. + 20h. + min.)/4 are shown in Fig 2 The mean annual rainfall (1937 to 1941) is 0 2 mm. The daily mean relative humidity (08h. + 20h.)/2, varies from 47 per cent. in December to 20 per cent. in June.

Table I shows the frequency of directions of wind, and of calms. The fig---
in Table II are abstracted from anemograph charts for a sample month, J---
1941 and 1942, and show the frequency and duration of diurnal and noctur---
southerly winds. It is seen that on the average for the year the prevail---
wind for the day is in the south west on considerably less than 1 day a mo---
In July southerly winds in general appear to blow about three times as m---
by day as by night and for about three times as long. The reason is that wh---
an air current from the south does reach Wadi Halfa it is considerably rein---
at night by the cooling of the desert air.

TABLE I

WIND DIRECTION AT WADI HALFA. THE FIGURES FOR EACH MONTH ARE PERCENTAGE FREQUENC---
OF DAILY MEANS, 1925 TO 1942 (CLIMATOLOGICAL NORMALS .... 1935).

Month.	N.	N.E.	E.	S.E.	S.	S.W.	W.	N.W.	Clm.
January	22·4	1·8	0·1	0·1	0·0	0·1	0·4	9·5	41·6
February	30·4	11·9	0·4	0·1	0·1	0·2	0·4	12·7	29·1
March	30·2	12·7	0·6	0·3	0	0·1	0·9	12·4	27·7
April	31·8	1·8	0·4	0·4	0·4	0·9	0·4	12·6	48·1
May	26·9	1·	0·7	1·3	0·5	0·4	1·1	12·4	41·1
June	31·4	13·4	0·1	0·1	0·0	0·9	1·6	15·8	31
July	17·3	5·6	0·0	0·	1·0	4·4	4·8	20·7	44·6
August	17·8	6·5	0·3	1·0	0·6	2·7	6	40·0	44·1
September	35·1	14·0	0·3	0·0	0·0	0·	0·6	1·8	28·9
October	79·7	16·9	0·6	0·5	0·2	0·2	0·	14·6	32·4
November	23·4	12·3	0·5	0·1	0·0	0·1	0·7	11·5	44·1
December	23·8	15·0	0·1	0·1	0·0	0·4	0·4	10·9	4·3
Mean	27·7	12·0	0·5	0·4	0·	0·9	1	14·6	42·7

TABLE II

FREQUENCY AND DURATION OF SOUTHERLY (S.S.E. TO S.W. BY W.) WINDS AT WADI HALFA
IN JULY 1941 AND 1942

Time.	Number of Occasions.	Duration in Minutes.			
		Total.	Maximum.	Minimum.	Year.
1941, day	15	839	165	4	96
,, night	4	155	50	18	39
,, total	18	994	165	4	35
1942, day	46	4,239	325	12	87
,, night	1?	1,568	248	20	111
,, total	6...	5,807	430	12	212

The Mosquito Fauna.

The following species were found in the area in 1942.

Anopheles (Myzomyia) gambiae Giles
"   "   *multicolor* Cambouliu
"   "   *pharoensis* Theobald
Theobaldia (Allotheobaldia) longiareolata Macquart.
Aedes (Ochlerotatus) caspius Pallas.
Culex (Culex) theileri Theo
"   "   *univittatus* Theo
"   "   *pipiens* s.sp *molestus* Forskål

As will be seen below, *A pharoensis* probably entered the area as a result of reservoir conditions, so that probably before the dam was built only seven species occurred. This number is not surprisingly small however since Wadi Halfa lies in the desert belt which divides the Ethiopian and Palaearctic Regions and there are few types of breeding places. The small fauna represents a mingling of Ethiopian and Palaearctic species.

Twenty two species of mosquitoes are common to the Ethiopian and Palaearctic Regions They comprise the twenty listed by Edwards (1941, p 450) *A gambiae* recently discovered in Egypt and *C p.molestus* recently identified in the Sudan. Of these twenty-two sixteen are found in Egypt and the Sudan, as shown by the records of Edwards (1941) Jobling (1938) Kirkpatrice (1925) and Salem (1933) Only eight of the sixteen species occur in the Wadi Halfa area. Three of these and several of the species not found at Wadi Halfa show an extensive gap in their distribution, large tracts of the Sudan separating their northern and southern areas Thus *A pharoensis* has only once been found between Kadaro near Khartoum, and Faras, which are 1 500 km. apart by river For some species such as *C postlipes* and *C antennatus* the gap is even greater

For a possible explanation of the present distribution in north-east Africa of some of the species now found in Egypt and the Sudan, reference must be made to the Pleistocene and recent geological periods. Nilsson (1940) considers that a series of alternating wet and dry phases with decreasing intensity have probably followed each other up to the present time in East Africa and Abyssinia and also that these phases have probably been contemporaneous with corresponding changes all over the world. We may assume therefore, that climatic changes in the northern Sudan have not differed greatly from those in Egypt which have been described by Ball (1939) It appears that during the middle and late Pleistocene Period (during part of the Great Ice Age of Europe) the rainfall of the present-day Eastern Desert of Egypt was rather heavy Probably also towards the end of the Pleistocene the desert west of the Nile was less arid than it is at present. By the end of the Pleistocene Period, possibly some 20 000 years ago desert conditions had set in in Egypt and also a great change in the Nile had occurred. The river had begun to bring

down immense quantities of silt from the central Sudan, some of which is deposited wherever the channel widened and the flow was reduced. Eventually the quantity of silt became reduced and the river assumed its present form in which the cataract region" (the stretch between Khartoum and Aswan) became an area of erosion between the areas of deposition above and below it

At the present day swamp-breeding mosquitoes, such as *A pharoensis* *C pocidipes* and *C antennatus* breed extensively at certain points along the Blue Nile where it has deposited silt in the form of large basins which remain flooded for several months each year   It seems possible that either of the Pleistocene conditions described above, the higher rainfall or the silt deposits may have provided perennial swamps necessary for the spread of the mosquitoes along the Nile Valley

In recent years the advent of reservoir conditions, by forming swamps beside the lower part of the Aswan Reservoir and by flooding the Faras basin has probably resulted in the return of *A pharoensis* to the Wadi Halfa area It is of interest that a specimen of this species from Faras is somewhat different in colour resembling Egyptian rather than Sudan specimens.

C theileri is unknown in the Sudan except near Wadi Halfa, although it is abundant in Eritrea (Lewis 1943)   It may have spread along the Red Sea hills in Pleistocene or later times.

The pool-breeding species, *A gambiae* and *C univittatus* can probably breed almost throughout the eroding cataract region " of the river

In the faunal map of Africa reproduced by Edwards (1941) the line separating the Ethiopian and Palaearctic Regions coincides with the boundary between Egypt and the Sudan where it crosses the Nile   Although these boundaries are for convenience represented by lines rather than broad bands, the Wadi Halfa area shows a striking relation between the zoogeographical boundary line and the known local limits of distribution of several species The known southern limits of *A multicolor* and *A pharoensis* in this part of the country are less than 1 km. from the boundary and the southern limit of the Palaearctic *C pusillus* is a few kilometres to the north at Balana (Musqra 1942).   The known southern limit of *C theileri* in the Sudan is in the Second Cataract, and the perennial northern limit of *A gambiae* appears to be at the general region of the Nile Valley

Aëdes aegypti has never been found at Wadi Halfa, probably owing to the low rainfall, unfavourable conditions of temperature and humidity and the abundant water supply which obviates the necessity for storage.

NOTES ON THE SPECIES

Anopheles gambiae —This species was first identified in the Wadi Halfa area in May 1941 (Lewis, 1942).   This finding, together with the fact that the species was formerly known as far north as Zeidab near Atbara (Evans, 1926) and was identified in Upper Egypt in 1942, might suggest that it had spread

northward into Egypt in recent years. There are however, several reasons for considering that this sequence of dates is a coincidence and that *A gambiae* has probably occurred in the Wadi Halfa area for many years. The annual reports of the Wadi Halfa Hospital record that railway employees were being given prophylactic quinine in 1919 and that in 1925 and several succeeding years malaria was contracted in the town The importance of the disease made anti mosquito measures necessary and these were begun in 1932. The existence of anophelines was first mentioned in 1931 although it is evident that they occurred before that year Except at Faras, where the conditions are peculiar and which is 33 km. from Wadi Halfa, the only anopheline seen by the writer between June 1942, and December 1943 was *A gambiae* It may be further noted that the identification of *A gambiae* caused no surprise to those engaged in mosquito control at Wadi Halfa and that KIREPATRICK (1925) wrote of *A gambiae* as An Ethiopian species which may occur in the extreme south-east corner of Egypt " (near the coast)

The approximate durations of the aquatic stages of *A gambiae* at different seasons at Wadi Halfa can probably be estimated by referring to the temperature curve in Fig 2 and to the figures for temperature in Table III This table

TABLE III

APPROXIMATE MINIMUM PERIODS OF DEVELOPMENT OF *Anopheles gambiae* AT WAD MEDANI IN 1941

Date of Hatching.	Period in Days.			Air Temp., C			Water Temp., C		
	Egg	Larva.	Pupa	Mean Max	Mean Min.	Mean *	Mean Max.	Mean Min.	Mean *
22nd Aug.	1	6	1	33 3	21 8	27 7	34 4	24 5	29 5
2nd Sept.	1	5	1	36 1	22 2	29 2	36 0	24 6	30 6
21st	1	9	1	35 2	22 9	30 6	37 2	25 1	31 2
9th Oct.	1	6	2	38 5	21 5	30 0	36 5	24 3	30 2
2nd Nov	1	5	1	39 4	23 1	31 3	35 0	24 8	28 9
19th	2	9	2	33 3	14 3	24 8	29 4	17 0	23 2
22nd Dec.	3	13	4	31 7	12 1	21 0	25 7	14 1	19 9

* (Max. + min.) /2.

summarizes the results of an experiment made at Wad Medani in which eggs less than 24 hours old were placed in a pool and the subsequent early stages examined daily at 08 00 hours. The insects were confined in a net half a metre square which suspended from floats and covered with mosquito netting at night to prevent the escape of adults The pool was 5 metres long 2 metres wide, and about half a metre deep and breeding outside the net was prevented by *Gambusia*. Periods are calculated from the first appearance of each stage It

is likely that at Wadi Halfa *A gambiae* can develop from egg to adult in 7 days in summer but may take a month or more in winter

In the area between Wadi Halfa and a point just south of Faras, the first anopheline larva found in 1942 was obtained in an irrigation channel at Ashkeit on 19th April and the last on 9th December in a similar breeding place at Dubeira. In 1942 and 1943 no anopheline larvae were found in January February or March in this area. Breeding appears to cease for some 4 months from about the middle of December till the middle of April presumably because pools do not exist for long enough. Since it is unlikely that the few adults present in December could live for 4 months in the dry desert air it is probable that the species normally dies out in this period in the area named.

Breeding begins in April with the rising temperature and tends to increase greatly in May when the falling river leaves many pools. It is curtailed in July by the rising flood and continues to only a limited extent, on irrigated land where water sinks rapidly into the porous soil.

The source from which *A gambiae* comes in April appears to be the Second Cataract. Anopheline larvae have been reported from pools in the Cataract in December and March, and the writer found a third stage larva of *A gambiae* in filamentous algae in an inlet among the rocks on 12th April 1943 a month that was about as cool as a normal March. It is likely that larvae exist in these large pools throughout the winter

In certain areas of southern Sudan, LEESON (1931) and DE MEILLON (1937) have shown that *A gambiae* passes the winter in warm places at low altitudes and invades cool high localities in the summer In the Wadi Halfa area there are no marked differences in climate from place to place because the range of altitude is small. The site of the wintering locality evidently depends on the presence of breeding places which exist for several months.

Anopheles multicolor —The many larvae of this species were found breeding in saline water in the canal at Faras West in 1942 and in pools in 1943.

Anopheles pharoensis —A somewhat dark specimen was bred from a larva in the Faras canal in June 1942.

Theobaldia longiareolata —This species is occasionally found breeding in cement tanks.

Aëdes caspius —Many larvae have been found in the Faras canal and in nearby pools. Females sometimes bit near the breeding place by day

Culex theileri —Larvae were found in the canal and in pools at Faras and occasionally in river pools in the Second Cataract and near Wadi Halfa.

Culex univittatus —This is a very common species which breeds chiefly in river pools and formerly bred in large numbers in the Faras canal. It is not known to bite man in the Sudan.

Culex pipiens s sp *molestus* —A mosquito common in the area has been provisionally assigned to this subspecies which is common in southern Europe Adults were seen biting man indoors in April and June, 1943. In April they bit both by day and night and males were seen indoors.

The species bred in saline pools at Faras and larvae are found in disused *atara* wells near Wadi Halfa. *C p.molestus* has been found in steamers where it was breeding in bilge water

THE CONTROL OF *A gambiae*

In addition to the protection of the inhabitants the control of *A gambiae* the Wadi Halfa area has the further aim of reducing to a minimum the ances of individuals of this species travelling to Egypt. The principal method achieving this object is to reduce the number of *A gambiae* to a figure proaching extermination.

There are four main reasons why this is possible without enormous expenditure. SOPER and WILSON (1942) give as one of the factors which make species radication feasible the Opportunity to eradicate the species in a sufficiently rge or isolated geographical area so that the periphery subject to reinfestation represents but a small fraction of the area worked. In the area between hor Musa Pasha and Faras about 98 per cent. of the periphery is desert. urthermore, the cold winter is unfavourable to *A gambiae* there is no rainy ason to provide abundant breeding places when the river pools are covered y the flood, and the prevailing wind blows from Wadi Halfa towards the ataract, the main possible outside source of *A gambiae*

Control measures in the area —The well-known methods—oiling Paris-green usting and filling and draining where practicable—are employed for river pools nd cultivation. Fortunately much of the irrigation stops before the beginning f the hot weather Paris green is mixed, 1 per cent. with Nile silt and distributed by hand. Some silt is deposited each year along the banks and provides n abundant handy supply of suitable diluent.

In the Faras basin control was effected by the use of *Gambusia holbrooki* nd Paris-green. It was found that anophelines did not breed if 150 fish were laced in the canal in the middle of August, Paris green applied till the middle f October and a few patches of dense filamentous alga removed by raking housands of young fish were to be seen in October and when the canal extended nto a swamp many of them invaded it and penetrated the shallow water at its dges. In June of the following year the numbers of fish in the canal were usually estimated at over 500 to the square metre or more than two million in he canal.

The Faras canal was very suitable for the use of *Gambusia*. The water ntered it entirely by seepage so that larger predatory fish could not enter No predatory birds were seen. The fish could be introduced during hot weather nd multiplied rapidly before the winter and before the swamp appeared. An idea of the probable rate of increase may be obtained from Table IV which hows the results of experiments at Wad Medani. Newly born fish were placed n pools 5 metres long by 2 metres wide and about half a metre deep and the ates of appearance of the first and second broods noted The average number

of fish used was seventy-eight. A permanent stock of *Gambusia* is maintained in a wide *matera* well at Wadi Halfa. Many of these wells are stocked by the owners with cat fish, *Clarias anguillaris* L., which are said to maintain the inflow of water by stirring up the mud at the bottom. The well and *Gambusia* is in a porous subsoil and therefore free from the carnivorous *Clarias*. The *Gambusia* were obtained from a stock at Wad Medani which was derived from a supply brought by KHALIL (1930).

A gambiae was never found in the isolated saline pools at Farm but they were treated with Paris green, and some stocked with *Gambusia* as a precaution and for the control of *A. multicolor*. BRUMPT (1942) states that *Gambusia*

TABLE IV

PERIODS BETWEEN BIRTH AND THE APPEARANCE OF FIRST AND SECOND BROODS OF *Gambusia holbrooki* AT WAD MEDANI

Date of birth (1943)			12th July	6th Aug	19th Sept	4th
Days before first brood			41	39	34	8
Days between first and second broods			19	14	17	—
A stage air temperature C	Before first brood	Mean	22.3	29.1	22.0	25
		Maximum	33.6	34.5	37.1	30.1
		Minimum	22.4	21.8	22.3	18.7
	Between first and second broods	Mean	27	29.6	29.2	
		Maximum	34.4	37.5	38.4	
		Minimum	1.8	22.5	21.7	

$$\frac{(8 \text{ h.} + 14 \text{ h.} + 20 \text{ h.} + \text{noon.})}{4} + 1.0$$

can resist a salinity of fifty-two parts of salt per 1 000 and reproduce at having up to from twenty to twenty five parts. SICAULT (1934) found that *G holbrooks* could be transferred to water containing at least 11 grams sodium chloride per litre (0·67 per cent. of chlorine) without causing the death or diminishing their capacity for feeding on mosquito larvae. One of the pools at Farm to which *Gambusia* were transferred and in which they appeared to flourish was found to contain 0·66 per cent. chlorine and in addition 0·63 per cent. sulphate. If the water should become too salt for *Gambusia* the coastal species, *Cyprinodon dispar* Ruppel, might prove useful. It does not

live in Nile irrigation channels (KING 1911) but lives indefinitely in tap water from the Nile at Wad Medani

Measures against tucanon —*A gambiae* might reach Wadi Halfa from the cataract by flying by drifting as larvae nr by carriage in sailing boats or cars. Larval drift is, however, improbable because numerous small fish live in the shallow edges of the river and cars and boats are few

It seems probable that in the past *A gambiae* spread northward in May and June each year by flying a few kilometres north of the Cataract passing through an aquatic cycle in the numerous residual pools south of the town and so moving northwards in increasing numbers until at the beginning of the flood some five generations had been passed   The frequency of northerly winds and calms presumably hindered the spread and in April 1943, the normal anti larval measures were changed with a view to obstructing the spread further   While inspections were continued throughout the area control was concentrated on a 6 km. stretch of river (its north end being 2 km. north of the Wadi Halfa railway station) and adjacent cultivation.   It was hoped that few or no anophelines would fly northward beyond this 6 km. stretch and that those which entered it would lay their eggs in the area where all breeding was controlled and would then be blown back to the south.   The year proved to be particularly favourable for the experiment because the river remained low later than at any time in the previous 30 years   At the end of April an outbreak of malaria occurred in the uncontrolled rural area of Abka and *A gambiae* was found breeding in many pools there and in other pools immediately south of the barrier   North of the barrier however where many similar pools were left untreated for observation, no anopheline larvae were found from May to July except in one pool in June and another in July   In the previous year numerous larvae of *A gambiae* had been found in many of these pools   At the time of writing (February 1944) larvae of *A gambiae* have been reported from only four pools in the whole area north of the barrier in the past 10 months   During the flood season the river rose unusually high and overflowed to the south of Wadi Halfa town, forming pools some of which remained for 3 weeks. No larvae appeared in them. It cannot be proved that the scarcity of *A gambiae* was caused by the barrier but this seems very probable and it is being extended into the Cataract in 1944

In addition to the Cataract, possible sources of infestation are other parts of the Sudan and Upper Egypt. Aircraft from the south are sprayed at the three aerodromes in the Khartoum area and trains from the south are sprayed on reaching Wadi Halfa. It may be noted that WHITFIELD (1939) during three years examination of aircraft landing at Khartoum found only two *A gambiae* in aircraft from the south. The necessity for spraying trains was shown by the finding in the month of December of a female of *A gambiae* biting in a north bound train in the desert 93 km. north of Abu Hamed.

Steamers from Egypt are sprayed by ' mosquito men who travel on

board. The Nile immediately north of the frontier has steep banks quite unsuitable for the breeding of *A gambiae* so that the mosquito is unable to fly into the area from the north.

Measures to prevent movement northward.—*A gambiae* has become so in the Wadi Halfa area that it is very unlikely to travel northward by road, steamers, or the very few sailing boats which ply As a precaution, however steamers are sprayed during the journey

THE CONTROL OF OTHER SPECIES.

A multicolor and *A pharoensis* are controlled by measures directed against *A gambiae* at Faras. *T longiareolata C theileri* and *C univittatus* are known to bite man in the Sudan and are not controlled. Although *Pura p—* does not kill culicine larvae its use is justified by the fact that *C univittatus* is the predominant culicine in most areas. *A caspius* does not appear to bite man except occasionally near its remote breeding places in the Faras area *C p molestus* is controlled near houses and in steamers.

SUMMARY

Owing to the position of Wadi Halfa on the Nile route the control of *A gambiae* is important to prevent it from travelling northward. Conditions affecting this and other species are described at some length because the Aswan Dam may be raised and produce further problems.

The mosquito fauna is discussed in relation to the zoogeographical position of the area.

Notes on each species and on seasonal changes in distribution of *A. pretoriensis* are given. It is probable that this species has occurred at Wadi Halfa for some years and that it winters in the Second Cataract.

Methods of control, particularly of *A gambiae* are described, with special reference to the possible effect of the prevailing wind.

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board. The Nile immediately north of the frontier has steep banks and unsuitable for the breeding of *A gambiae* so that the mosquito is unlikely to fly into the area from the north.

Measures to prevent movement northward.—*A gambiae* has become so rare in the Wadi Halfa area that it is very unlikely to travel northward by the steamers, or the very few sailing boats which ply As a precaution, boats steamers are sprayed during the journey

THE CONTROL OF OTHER SPECIES.

A multicolor and *A pharoensis* are controlled by measures directed against *A gambiae* at Faras. *T longiareolata C theileri* and *C univittatus* are known to bite man in the Sudan and are not controlled. Although Paris green does not kill culicine larvae Its use is justified by the fact that *C. ......* is the predominant culicine in most areas. *C caispus* does not appear to bite man except occasionally near its remote breeding places in the Faras area *C p molestus* is controlled near houses and in steamers.

SUMMARY

Owing to the position of Wadi Halfa on the Nile route, the control of *A gambiae* is important to prevent it from travelling northward. Conditions affecting this and other species are described at some length because the Nile Dam may be raised and produce further problems.

The mosquito fauna is discussed in relation to the zoogeographical parts of the area.

Notes on each species and on seasonal changes in distribution of *A. gambiae* are given. It is probable that this species has occurred at Wadi Halfa for years and that it winters in the Second Cataract.

Methods of control, particularly of *A gambiae* are described, with special reference to the possible effect of the prevailing wind.

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YELLOW FEVER IN THE RECENTLY INOCULATED

BY

MOUNTJOY ELLIOTT M.A. M.D., M.R.C P I., CAPT R.A.M.C

In October 1942, during a Parliamentary debate, some facts were released about the effects of yellow fever vaccine on Service personnel inoculated in this country and in America. At least 135 000 people received preventive inoculation between January 1941 and June, 1942. The Secretary of State for War said that three cases of yellow fever had occurred among British and Allied troops since the commencement of war and as these patients came under my care in a West African military hospital, I thought it would be of interest to report them.

Clinically the cases would be classified as severe and two of the patients died within 4 days of admission to hospital. All three men had received preventive inoculation at least 2 years before developing yellow fever. A summary of the main clinical signs and symptoms is given in the table and the 24-hour temperature chart for Case I is reproduced.

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The following is a summary of the autopsy findings and animal inoculation experiments carried out by Lt.-Col B G MAEGRAITH Area Pathologist.

Case I

Lieut B (Polish Forces) aged 32. Inoculated with yellow fever vaccine September 1941   Admitted 5 1 42 after 2 days illness and died on 8.1.42 Subpleural and retroperitoneal haemorrhages   Massive haemorrhages in both lungs. Kidneys and spleen showed congestive enlargement. No malarial parasites or pigments found in any of the tissues examined. Submucous
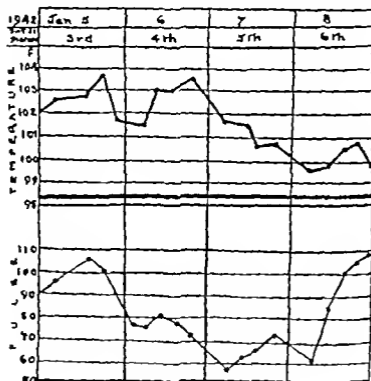
Case I   (Lieut B)

haemorrhages into the cardiac end of the stomach and the terminal ileum. The brain was uniformly congested and there were punctate haemorrhages into the floor of the fourth ventricle. The biscuit-coloured liver weighed 1 650 grammes, was reduced in size, and was fatty and friable   Microscopically there was a fatty degeneration and desquamation of the renal tubular epithelium. Councilman lesions of the cytoplasm of the hepatic cells were noted and eosinophilic intranuclear inclusion bodies were seen in most sections. Macerated portions of liver tissue were inoculated into two *Macacus rhesus* monkeys and

intracerebrally into two white mice without adverse results Urine sediment
was inoculated into the peritoneal cavity of a guinea pig The animal remained
healthy and no leprospira were isolated

TABLE.

Faget's Sign.	Jaundice	Enlarged Spleen	Vomiting	Urine	Weil Felix Test.	Cerebro spinal Fluid	Blood
+++	++	+	++ Terminal Black Blood +++	Albumin +++ Blood +++ Granular casts +++ Epithelial casts +++ Pus + Oliguria.	—	Sterile Clear Reduced ten sion Kahn — 5 w b.c. per cu mm.	No malarial parasites. Sterile Kahn — 9 450 w b.c. per cu. mm Polymorphs. 93
+	+	—	++ Blackish Blood +++	Albumin +++ Blood +++ Granular casts +++ Epithelial casts + Pus +++ Oliguria.	—	Sterile Clear Normal ten sion. 3 w.b.c. per cu mm. Protein 25 mgm. Chlorides 480 mgm. Kahn —	No malarial parasites. Sterile Kahn — 8 125 w b.c. per cu. mm. Polymorphs. 79 5% Urea, 144 mgm.%
+++	+	—	+ Blood +++	Albumin +++ Blood +++ Granular casts + Pus + Oliguria	—	Not done	No malarial parasites Sterile. Kahn — 3,200 w b.c. per cu. mm. Polymorphs. 69% Urea, 145 mgm. Sugar 112 mgm.

Case 2.

Sgt. Bl. (British) aged 35 Inoculated with yellow fever vaccine November
1940 Admitted 10 2.42 after 3 days illness and died on 13 2.42. The results
of the autopsy findings and the inoculation experiments were similar to Case 1

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 16th November, 1944, at 3 p.m

THE PRESIDENT,

SIR HAROLD SCOTT, K.C.M G , M.D , F.R.S.E.,

in the Chair

PAPER.

AMOEBIASIS WITH SPECIAL REFERENCE TO TREATMENT

BY

A. R. D ADAMS M.D., M.R.C.P., D.T.M.

Liverpool School of Tropical Medicine.

At present very large numbers of men overseas are being exposed to infection with *Entamoeba histolytica* under conditions particularly favourable to the establishment of the parasite in them. Some of these men suffer from gross clinical attacks of amoebic dysentery, others give no such history but are found to be infected on routine examination of the stools. Of these latter cases many complain of no symptoms attributable to their infestation, but others suffer from a variety of mild and indeterminate digestive and abdominal disturbances which might be attributed to their amoebiasis. These cases constitute the carriers of the infection and there are two schools of thought regarding the nature of their parasitization One, represented largely by Continental workers during recent years (REICHENOW 1937 WESTPHAL, 1938), postulates that in these cases the parasites live in the lumen of the gut, and only under certain conditions do they invade its wall and produce lesions. This school in short, considers the infection normally a commensal one which may become pathogenic. The other school holds that every *E histolytica* infection is pathogenic and that lesions are invariably present. They consider that it is the

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size, the site the number and the distribution of colonies of *E. histolytica* in the large bowel which determine the patency of the clinical manifestation of their presence In support of this view there is experimental evidence that no strain of *E histolytica* is non pathogenic to animals, although there may be some variation in the virulence of different strains of the parasite. The virulence of strains in experimental animals has been enhanced by rapid subpassage and massive infection (MELENEY and FRYE, 1937 FAUST and SWARTZWELDER, 1935), and it may be that where rapid subpassage occurs in a man for example under present conditions in the field, a similar enhancement of virulence for him may result.

Whichever view one favours—that assuming normally a lumen infection by a parasite which may alter its habits, or that which regards the parasite invariably as pathogenic from the start—it seems to me improper to neglect a detected infection with *E histolytica* until clinical manifestations make their appearance. To do so is to condemn many patients to subsequent unnecessary ill-health with the possible development of a major disaster such as an amoebic liver abscess. Furthermore, such patients, under insanitary conditions, are liable to disseminate the infections to others. I therefore think they should be regarded as latent cases requiring early treatment and should not be shelved or ignored.

To discover these infections necessitates routine repeated stool examination. While this measure is obviously impracticable in the case of every man who has served abroad, nevertheless it can and should be done in the case of all men under observation in hospital whatever the reason for their presence there The number of pathologists and technicians really competent to examine stools for protozoal infections is remarkably small. There is a tendency for the professional microscopist to think that after a cyst or two, and possibly a few amoebae, have been pointed out to him the diagnosis of the presence or absence of an intestinal protozoal infection is a simple matter This is by no means the case and, as pointed out by DOBELL (1917), a full-time apprenticeship of some months followed by regular practice is necessary for real efficiency in the direct microscopical examination of stools. It is the lack of this specialized training which is responsible for the unfortunate misdiagnosis so frequently encountered in the investigation of cases of colitis. There appears to be some general agreement that repeated microscopical examination of the stools is a satisfactory method of detecting an *E histolytica* gut infestation, and that employment of the various concentration techniques and cultural methods available does not so materially increase the chance of finding parasites—when too scanty to be seen microscopically—that they are worth the time spent on them. Craig's complement deviation test is rarely done in this country in view of its complication and of the difficulty in preparing a satisfactory antigen.

Before turning to the present treatments of amoebic dysentery it may be profitable briefly to consider the stages by which they have been arrived

at Nearly three centuries ago ipecacuanha was introduced to Europe from South America, and it then became widely used with varying enthusiasm for the treatment of the dysenteric disorders For example, in 1858 DOCKER recorded the remarkably successful treatment of dysentery among troops in Mauritius with large doses of the drug (60 to 90 grains by the mouth two or three times daily) and the resultant fall in the annual death-rate from the disease among them his successful method of giving the drug re-popularized its use in India. In 1817 PELLETIER and MAGENDIE had isolated the alkaloidal vomiting principle, which they called émétine, from ipecacuanha, but subsequently ipecacuanha was found to contain five alkaloids three at least of which had been extracted together by PELLETIER and MAGENDIE, who thought them a single entity It was not until the early years of this century after *Entamoeba histolytica* had been identified and its significance as a pathogenic agent had been recognized (SCHAUDINN 1903) that ipecacuanha began to be employed on a rational basis as a specific against the parasite. Some differences of opinion then arose, as to the relative values of ipecacuanha and of ipecacuanha *sine emetina* in the control of acute amoebiasis. VEDDER (1911 and 1912) suggested after a demonstration *in vitro* of its amoebicidal action on free-living amoebae of the limax type, the employment of the alkaloid emetine, in human amoebiasis. As a sequel, ROGERS (1912) unequivocally showed the specific action of hypodermic injections of the soluble salts of emetine in intestinal and liver amoebiasis. At the onset of the first World War emetine hydrochloride by injection had become firmly established as the most rapidly effective method of control of acute amoebic dysenteric infections of the gut and of amoebic lesions in other tissues, a position from which it has not so far been displaced. But it was fully recognized and has since been repeatedly confirmed that emetine injections alone would not eradicate a gut infection in man in more than about one-third of those cases treated with it (CRAIG 1934 puts the figure between 10 and 15 per cent.) so further efforts were made to find other preparations of the drug more certain in achieving this end.

In 1915 DU MEZ prepared a new compound, a double iodide of emetine and bismuth, suitable for oral administration. The following year DALE (1916) used E B I. to treat ten cases of amoebic dysentery previously given emetine injections without producing sterilization. He considered six of these probably to have been cured by the course, two were not cured, and two were unable to withstand the full course of treatment owing to the nausea, vomiting and diarrhoea induced by the drug DALE concluded that the drug was of considerable therapeutic value and also might be of use prophylactically Low and DOBELL (1916) a few weeks later confirmed DALE's opinion of the therapeutic value of E.B I after using it in three cases of amoebiasis All three were sterilized of their infections, and Low and DOBELL were convinced that E.B.I. by mouth was far more efficacious than emetine by injection in sterilizing the gut infections of latent cases. Since then E.B I. has been extensively used

and today it is regarded by many English workers as a sheet-anchor in the eradication of *E. histolytica* infections. Among further preparations of emetine for oral administration which have been tried and which still have some adherents are emetine periodide, introduced by MARTINDALE in 1923, and auremetine, a combination of the periodides of emetine and the dye auramin, introduced in 1926 by WILLMORE and MARTINDALE.

In spite of the choice of emetine compounds available, it appears that most cases of amoebiasis are not sterilized of their infections by the emetine compounds alone in whatever form and for however long they may be given.

In this situation it becomes necessary to seek other drugs possessing therapeutic action on the parasites and among these bismuth salts are reputed to hold a place. Bismuth subnitrate has long been used in the treatment of amoebiasis and, in particular JAMES and DEEKS (1925) considered it to be effective in eradicating gut infections with *E. histolytica* in Panama when given in very large doses, either together with emetine or even alone. There is by no means universal confidence in the virtue of bismuth salts in this infection though they are still much used.

Sundry synthetic arsenical compounds for oral administration have at various times been advocated as exerting a therapeutic action on intestinal amoebic infections. Of these, stovarsol and carbarsone have been most employed in the past and are in use today. MARCHOUX (1923) reported a rapid action of stovarsol both on *E. coli* and on *E. histolytica* infections and claimed to have sterilized three cases of the former and ten cases, both acute and chronic, of the latter parasitic infection by this drug alone, albeit the treatment had to be repeated in some of them. Others since then have not been so remarkably successful with stovarsol. Carbarsone, advocated by ANDERSON and REID (1931) as a specific for amoebiasis, was originally prepared by EHRLICH. It is chemically is somewhat similar to stovarsol, but is said to be less toxic. Its use in dysentery is alternative to though American workers believe it to be more effective than, stovarsol, and they have given it not only orally but as a retention enema (ANDERSON and REID 1934), 200 c.c. of a 1 per cent. solution in 2 per cent. sodium bicarbonate being employed for this purpose.

There remains one other type of drug which is generally believed to possess therapeutic properties in cases of amoebiasis. Sodium iodoxyquinol-sulphonate, containing just under 30 per cent. of iodine, was introduced into the treatment of amoebiasis by MÜHLENS and MENK (1921) and marketed under the trade name yatren 105 (now chiniofon, B.P.). It can be given by the mouth or can be used in enemata, and is almost non-toxic. MÜHLENS-RUGE and MORRIS (1925) first reported on its combined oral and rectal use in this country and stated that it gave satisfactory results in three cases, which were apparently sterilized of their infection, but less satisfactory results in a fourth case incompletely treated with it which was subsequently cured by ERL. Chiniofon under various trade names has since been accepted as being of

considerable value in the therapy of amoebiasis and is still much used, CRAIG (1934) would appear to regard this drug alone, when given both by the mouth and in enemata for 8 to 10 days as adequate to sterilize most cases of amoebiasis if not of long standing and with a history of many relapses. Since chiniofon was introduced, two somewhat similar preparations have made their appearance. Vioform (1933) which contains nearly 40 per cent. iodine, and diodoquin (HUMMEL 1939) containing over 60 per cent. of iodine, can be given by the mouth, but are too irritant for rectal use and these compounds are advocated in the treatment of amoebiasis, particularly by American workers.

On considering the value of these various drugs each of which has had vigorous protagonists it is evident that none of them can be regarded as infallible in sterilizing a gut infection with *E histolytica* though there is evidence that each of them may contribute something to this end.

Nevertheless during the latter part of some years personal experience of amoebiasis I must confess that until very recently I had thought that the treatment of a gut infection with *E histolytica*, in all but an infinitesimally small proportion of cases, had become a matter of simple routine, If the case were acute a few preliminary injections of emetine arrested the attack. Then a three weeks blunderbuss assault on the parasites with auremetine, stovarsol and bismuth submitrate by mouth, and retention enemata of chiniofon, or with any other combination of similar drugs eradicated them with almost unfailing regularity Of many hundreds of cases treated in this country I can recall but very few requiring a second or a third such course and only one which, as far as I could follow it, proved completely refractory to treatment In my view the actual preparations of the drugs do not very much matter I think it was the period of time over which a number of drugs were given in concert that produced the extremely satisfactory results obtained. For example, I myself have used auremetine because I found it easy to administer being unlikely to cause nausea and vomiting but I do not hold any strong opinion on its superiority or otherwise to E.B L

Last year I first encountered a batch of cases just arrived from the India and Burma theatres. These cases, some thirty in number on arrival were bedridden, emaciated and passing frequent stools containing much blood and mucus and many amoebae They proved largely refractory to the usual 3 weeks' course of treatment in that, although their general condition improved remarkably during it, they were not sterilized of their infections and some actively relapsed within a few days of its completion. Repetition of the course on several occasions has now produced sterilization of the infection in many of them but others are still being hopefully treated. A similar state of affairs has obtained with many other cases since arrived from these areas in particular It is only after prolonged repeated and intensive courses of treatment with varying combinations of drugs that a proportion of them so far have been cured of their infections the residue are still infected despite any course of treatment I have

been able to devise to date. These treatments have included among others oral administration of sulphaguanidine and of sulphasuccidine, and the oral administration of these compounds in cod liver oil, and of rectal retention enemata of mepacrine hydrochloride. All produced temporary amoebiasis, but none showed evidence of specific action on the causative parasite.

On inquiry I am struck by certain factors which appear to me to differentiate these intractable cases from the more amenable cases I had previously encountered. The individuals have been treated with repeated massive (twelve injection) courses of emetine, and in many no other treatment has been given for adequate periods. It is usual to find that they have had from five to 300 injections of emetine hydrochloride for their recurring dysenteric attacks over a period of from 6 months to 2 years or more, and that each successive course has been followed by a lessening period of latency before the inevitable relapse occurred. There appears to have been a lack of appreciation of the fact that emetine alone will only occasionally eradicate an amoebic dysenteric infection, and this in spite of the teaching of every recognized authority since the beginning of the last war. I feel it should be better recognized by the profession as a whole that twelve injections of emetine are a maximum, and not a routine, number for the control of acute intestinal amoebiasis and that a very few injections will usually arrest the acute attack. There seems to me no valid reason for continuing the injections beyond this point, and none for repeating them unless the infection is allowed to run riot.

There are two explanations of the intractability of the infections. Either these men are a selected population infected with an unusually virulent and resistant parasite or as I myself think, they have been excessively dosed with emetine long after it should have been plainly evident that this drug would not sterilize them and their parasites as a result have become even more resistant to the action of the drug.

It may well be asked why they do not readily yield to the assortment of other drugs to which they can be subjected. The answer to this question I can only supply by suggesting that at present emetine in one form or another is the essential basis of any uniformly effective treatment of intestinal amoebiasis, and that an infection made resistant to this drug is the less amenable to any other of the drugs now available.

The unpleasant fact remains that there is an ominously large number of chronic relapsing cases of amoebiasis returning to this country and unless more satisfactory treatment is devised that number is going to swell to serious proportions with a further legacy of chronic post-dysenteric colitis in Ministry of Pensions hospitals in the post-war years.

I find it extremely depressing to encounter this influx of new cases of intractable colitis now steadily adding to the number still left after the last war. The urgency of the problem of the treatment of amoebic dysentery and of the avoidance of its sequelae seems to me an even more serious one than

the determination of the optimum method of treating malaria. Malaria in the individual usually is easily dealt with and within reasonable time is over and done with. This is by no means the case with severe amoebiasis which, even if ultimately eradicated, may leave the individual subject to chronic ill-health and unable to earn a normal living with the accompanying mental, moral and physical deterioration such a state entails

It seems to me that the whole matter of the treatment of amoebiasis needs a new approach, and that there is little likelihood of progress until some fundamental experimental work is done from the chemotherapeutic aspect. The intracaecal infection of animals has done much to facilitate the maintenance of strains *in vivo* and with this technique it should be possible to make the investigation of the action of drugs on *E histolytica* less empirical and more scientific. English workers have not been conspicuous in the field of amoebiasis during the last dozen years and the time is opportune for them to get to work now that the raw material, unhappily is so plentiful.

SUMMARY

1 It is advisable to regard every intestinal infection with *Entamoeba histolytica* as pathogenic.

2. It follows that every detected infestation should be eradicated as early as possible in the interests of the patient. An additional incentive to eradication is the possibility of the dissemination of the infection to others.

3 Emetine has a more specific action on this infection than any other available drug

4 Emetine alone by injection will not sterilize an infection in more than a minority of cases if it is given unwisely the infection becomes resistant to the drug in all forms and less amenable to any treatment.

5 Therefore the use of emetine by injection should be restricted to the control of clinically acute manifestations and the minimum amount necessary to achieve this end should be given.

6 The eradication of a gut infection must be attempted by the use of a variety of drugs including emetine preparations all of which have some action on the infection. These drugs should be given together on the grounds that their combined effect is greater than that of any single drug alone and they must be given as early as possible over an adequate period. Experience has indicated that a 3-week period of such treatment ensures a very high proportion of cures in cases not previously repeatedly treated subcuratively

7 When unsuccessful in sterilizing the infection the course, with, possibly changes in the preparations of the drugs used, should be repeated on several occasions as requisite, and without delay until the infection is ultimately eradicated.

8. There is an urgent need for fundamental investigation in order to make the testing of new drugs in amoebiasis more scientific and less empirical than it has been in the past.

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DISCUSSION

Lt.-Col. W. H. Hargreaves. In his excellent address, Dr. ADAMS has drawn attention to the severity of the problem which we are facing now[,] and which seems likely to confront us in this country often the war. This problem will undoubtedly increase if it is true that every individual whose stools are found to contain *Entamoeba histolytica* should be regarded seriously and given a course of treatment which at present in the case of a soldier on admission to hospital for several weeks—for I doubt if it would be found practicable in war time to hospitalize every so-called healthy carrier until his stools are pronounced clear. Furthermore, in my experience, on examination of their stools after a month has elapsed not all such cases have been found to be cleared by a 3 weeks combined treatment.

It seems fantastic in these days of brilliant progress in other fields of therapy that emetine, whose parent, ipecacuanha, was employed 3 centuries ago in the treatment of dysentery should still be the most potent drug at our disposal in the treatment of amoebiasis and that there should still be no single drug which is satisfactory when given alone. When I learned that Dr. ADAMS was going to speak on this subject I hoped that today we might hear of a new form of chemotherapy but our prescription still has to be rep. omnes.

At the military hospital where I have been working we have to contend with patients suffering from chronic amoebiasis, most of whom have been invalided home and many of whom have already proved refractory to treatment in other hospitals in this country. Often, as Dr ADAMS has said, these patients are wasted and bedridden with persistent diarrhoea, their stools containing much blood and mucus and many amoebae. They have usually been under almost continuous treatment in hospitals for many months and sometimes for as long as 2 years. One sometimes obtains a history of initial treatment with injections of emetine alone, particularly in cases dating from 1942 in Burma, where conditions appear to have been very difficult. Some men for instance, had to attend as out-patients for injections of emetine at hospitals whose beds were full.

This is not always the story however and some of our worst cases were given full standard courses of treatment from the start, receiving emetine E.B.I. chiniofon retention enemata and stovarsol. I would not rush to criticize the way in which emetine has been used by the medical officers who have had to treat some of our patients. I have seen them become acutely ill during the course of a 21 days blunderbuss treatment, and have myself in desperation given injections of emetine in order to relieve them.

My feeling is that in the case of many of these chronic patients it might have been wiser to have invalided them home sooner. Occasional bowel upsets, presumably infective in origin are common enough in the healthiest of Europeans out East, and it appears to me that secondary infections may well play a part in preventing the recovery of a diseased bowel.

I would like to say a few words about the general treatment of these cases Firstly, I feel that they should be given as full a diet as possible. Secondly very great patience has to be exercised in treating them. A cheerful ward sister is a great boon as, psychologically, men who have been in hospital continuously for many months and had repeated courses of treatment without success tend to reach a pathetic state of depression and to give up hope.

Many of our patients have formed their own ideas about the correct treatment and many ask for emetine injections as soon as they reach us, and are most sceptical about any new form of treatment. A dramatic improvement on the part of one of the bad cases has a most uplifting effect on the morale of the whole ward and, I might add, on the morale of the medical staff.

Until March of this year the well-known standard treatment had been used—six injections of 1 grain of emetine daily followed by E B I together with chiniofon retention enemata for 12 days, and lastly stovarsol or carbarsone 4 grains twice daily for 12 days. In March, General BIGGAM asked us to compare this with Dr ADAMS 3 weeks course, and we did not find that the Liverpool treatment had any advantage. We treated a series of seventy cases, fifty four of whom had been invalided from India, giving thirty five the Liverpool course and thirty five the standard course. Of these seventy forty-eight were apparently cured and of the remaining twenty two sixteen

of which were from India, twelve improved but relapsed after a few days or weeks and ten were unchanged.

I am sorry to say that sixteen of these twenty two patients who were not cured by one course had been given the Liverpool treatment. My feeling is that these figures may be unfair to Dr Adams as there is rather a tendency for a medical officer when admitting two cases to try the newer form of treatment on the severer case. I think, though, that in the Liverpool course it can be a bad thing to allow patients to leave bed on the alternate days when retention enemata are given. In this 21-day course bismuth carbonate is given throughout, with suremetine on the odd days and stovarsol, together with chiniofon retention enemata, on the even days.

In addition to the drugs described by Dr Adams, with the except of diodoquine and mepacrine, we have tried extract of kurchi bark, bismuth iodide and also stilbamidine without success. We have also tried sulphaguanidine and succinyl sulphathiazole (sulphasuxidine). The latter in particular relieves the symptoms in many cases but does not appear to affect the amoebae.

In May of this year we had two patients who were critically ill, and one of them died. He had had intermittent diarrhoea since July 1943 in East and had been admitted to an E.M.S hospital in this country in January. It was thought there to be suffering from bacillary dysentery and improved on sulphaguanidine but relapsed after a few days, when amoebae were found in his stools. He was transferred to us as he had not responded to twelve injections of emetine. On admission he was 4 stone under weight and was passing six stools a day with blood and mucus and numerous amoebae. He complained of almost persistent colicky abdominal pain. His abdominal wall was rigid, the liver edge was palpable and tender and there was tenderness along the whole course of the colon which appeared to be thickened. There was a leucocytosis of over 15,000 W B C per c.mm., but screening of the diaphragm revealed no abnormality. This patient went downhill in spite of treatment. He was transfused, given two courses of Liverpool treatment and six injections of emetine but developed signs of general peritonitis with free fluid in the abdomen and died.

At postmortem examination the peritoneal cavity contained turbid fluid. There were numerous adhesions and loculi of thick pus. The wall of the terminal 6 inches of ileum and the whole of the large bowel was dark in colour, thickened and rigid and the mucous membrane was almost entirely necrotic. The necrotic process extended in places deeply into the muscular layer of the wall and numerous perforations were present, some being sealed off by the omentum. The liver was moderately enlarged and contained two small abscesses.

Meanwhile the second of these cases was also going downhill. He had been invalided from India, where he had been in hospital almost continuously since 1942, and had had repeated full standard courses of treatment from the

start. He had arrived home in December, 1943 but in spite of two courses of treatment in another hospital he had shown no improvement and was transferred to us in April.

He again was cachectic and pyrexial with persistent abdominal pain and some twenty foul stools daily, containing blood and numerous amoebae. His colon was exquisitely tender and appeared to be thickened throughout its length. Sigmoidoscopy was impossible owing to pain, but we obtained a view of the lower part of his rectum, which is really all that one needs in these cases. It was almost covered with patches of ulceration, the intervening areas of mucous membrane appearing injected and oedematous. There was a leucocytosis of 20 100 W B C per c.mm. In spite of six daily injections of 1 grain of emetine, followed by a Liverpool course, his condition deteriorated We asked the surgeons to see him with a view to appendicostomy or ileostomy, but they did not think that he would survive an operation. He appeared to be moribund and required repeated administrations of morphia to relieve his pain. At this stage he was seen by General BIGGAM, who suggested that we should try penicillin.

We did so giving him an initial dose of 100 000 units intramuscularly, followed by 33 000 units 3-hourly up to a total of just over 1 000 000 units, 24 hours after starting the penicillin he was free from pain and apyrexial. After 2 days he passed a formed stool for the first time in 2 years. He rapidly put on weight, but amoebae were still present in his stools and after 2 weeks there was a recurrence of diarrhoea with blood and he was given a second course of penicillin—this time 2,000 000 units Again his stools became normally formed and we found on sigmoidoscopy that as far as we could see his sigmoid and rectum were now normal This time the instrument was passed without any difficulty We then sent him for a month's convalescence, and though he felt fit on his return amoebae were again found in his stools. We then gave him another course of Liverpool treatment, after which his stools were negative and he is now at a convalescent home again for another month. He has put on 4 stone in weight. We have no doubt that this patient's life was saved by penicillin and since treating this first case we have given it in a dosage of 2,000 000 units to other severe cases again with dramatic responses

We have not been able to demonstrate in the laboratory that penicillin has any effect on *Entamoeba histolytica* but it seems reasonable to suppose that its action in these cases is to combat secondarily infecting organisms. Secondary infection must surely play a part in these severe refractory cases with palpable tender colons in whom gross ulceration can be seen on sigmoidoscopy Any of the bacteria present normally in the faeces can gain access into the bowel wall through the ulcerated mucosa, including numerous strains of streptococci and staphylococci, many of which are penicillin sensitive. With the object of combating some of the penicillin resistant organisms we now give a course of sulphaguanidine in conjunction with it, and usually give a total dosage of 60 grammes by mouth. Our course of treatment now for these

severe refractory cases consists of sulphaguanidine by mouth and penicillin intramuscularly followed by a standard anti-amoebic course lasting some 3 weeks. We have seen marked improvement on sigmoidoscopy after penicillin given alone. When they are fit for convalescence we send our patients away for a month, after which they return for another sigmoidoscopy and examination of the stools—we use a concentration method and examine their specimens taken on alternate days. In one case we have had to repeat the second part of the course, but we feel that the attack on secondary infection has rendered the severe refractory cases more amenable to treatment, and those drugs which have a specific action on *Entamoeba histolytica*.

May I mention one further case I recently treated with penicillin: a girl who had 18 months history of ulcerative colitis, which was diagnosed by sigmoidoscopy and barium enema. I gave her a course of sulphaguanidine and penicillin, and 2 weeks after finishing the course I sigmoidoscoped her again and found that the mucous membrane of her rectum and sigmoid was normal. She may just have been a lucky case, but from this experience I think it possible that penicillin may prove helpful in the treatment of chronic ulcerative colitis.

I hope you will forgive me if I end with a few words in comic relief. I was asked recently to review a year book of modern treatment for 1944, and was amused to read the following at the end of the chapter on ulcerative colitis. " To sum up the treatment of this disease, the most important points are the following Number one, make sure of the diagnosis. It is very easy to miss a case of amoebic dysentery and treat it as ulcerative colitis when all that is needed is a few doses of emetine."

The President Sir Harold Scott First, I would like to congratulate Dr. Adams and Colonel Hargreaves on a most interesting exposition of their views on and experiences in the treatment of amoebiasis. As Dr Adams has said in his paper the treatment of this condition is largely the history of the use of ipecacuanha—and what a fascinating history it is. Dr Adams, in the time at his disposal was able to give us but the barest outline. May I add one or two more facts for my time too is limited by our rule to 10 minutes ?

Ipecacuanha was mentioned as long ago as 1625 nearly 320 years ago in Purchas's *Pilgrimes* It was brought to Europe from Brazil in 1658 and was used in India from 1680 onwards. Ipecacuanha was the so-called " secret remedy " used by Helvetius in 1680 and he was much helped and encouraged by Louis XIV who you will remember was the one to subsidize and later to purchase Talbot's Secret Remedy for Ague —cinchona.

As Dr Adams has told us, Scott Locker gave it in large doses to his patients in Mauritius he reported this in 1858 but he had been using it for the previous 10 years. He, however was not the first for Parkes in India had been giving as much as 60 grain doses in 1846 Nevertheless, it was

still being spoken of as a ' new remedy for the treatment of dysentery" in 1855 Three years before this in 1852, Hospel, a French physician, was giving it for hepatitis and liver abscess, and between 1852 and 1862 Delioux and Savignac treated many patients in the Naval Hospitals of Rochefort and Toulon and recorded that ipecacuanha was as specific for dysentery as quinine for malaria" A quarter of a century later Norman Chevers and Maclean were giving it empirically in hepatitis to prevent formation of an abscess and it remained empirical till Rogers in 1902, by showing that hepatitis was secondary to amoebic dysentery changed the empirical to a rational use.

Next, to prevent vomiting the emetine was removed and *ipecacuanha sine emetine* was tried, but proved disappointing and only later in 1911, as Dr Adams has told us, did Vedder show that the benefit of using ipecacuanha in dysentery was due to the alkaloid which had been so carefully abstracted

Dr Adams and Colonel Hargreaves have spoken of other drugs One or two I hoped to hear about were not mentioned. If either of them has tried it one would like to hear his experiences with conessine, the alkaloid from kurchu or telichery bark, *Holarrhena antidysenterica* also the Chinese remedy the seeds of *Brucea javanica* which seems to have met with such success in some hands. This, under the name of ya tan tzu, has been used in China for 180 years at least and nearly 40 years ago in 1905 that enterprising firm, Burroughs Wellcome & Co had a tabloid prepared from it under the name kŏ-sam, a synonym for ya tan tzu I do not know whether the tabloids were successful probably not, for two reasons. First, because one does not hear of the tabloids now second, one would doubt it on *a priori* grounds because the best results seem to be obtained only when the whole seeds in their capsules are taken. Liu reported success with it in 1937 and quite recently another very favourable report by Wu has appeared in the *Chinese Medical Journal* for December 1943 It is given in the following way On the 1st 3rd and 5th days twenty seeds in their capsules by mouth three times a day and on the 2nd, 4th and 6th days twenty seeds are soaked for 2 hours in 200 c.c. of 1 per cent. NaHCO₃ and administered as an enema to be retained after a washout. The clinical results were noted and correlated with sigmoidoscope appearances in twenty five patients whose ages ranged between 11 and 67 years, some acute (less than 1 month) and some chronic (up to 7 years duration) He says In nineteen of the twenty five, symptoms cleared in 2 to 5 days and the entamoebs could no longer be found in the stools. The local lesions healed in 5 to 10 days in six patients and within another week in seven more.

Three others are recorded as improved, symptoms abated and the entamoeba disappeared but in one patient in poor condition the symptoms recurred 3 weeks later another a syphilitic, feeling better refused further treatment the third suffered from bacillary dysentery also and the local lesions did not clear up till this, too had been treated. Three are returned as failures, but in one of these the bowel symptoms cleared up though

the fever continued, a liver abscess was found and emetine given. The patients have been followed up and Dr Wu reports. Five had remained well, but had left hospital only a few months. Another had been out 18 months, two others for more than 2 years, and eleven for more than 3 years and had remained well. Two relapsed 3 and 6 weeks respectively after discharge, one of these was again successfully checked by ya tan tzu. Toxic effects were negligible. Eight patients complained of nausea and four vomited a few had abdominal discomfort or actual pain (but this may have been due to the dysentery as much as to the drug).

I do not know if these seeds are difficult to obtain. If not, I wonder if any of the clinicians here can tell us his experience of their use, or why a drug, apparently so successful in some hands at least, has not been more widely tested.

Brigadier Robert Priest In the Western Command we have been equally interested in studying the problem of the treatment of amoebic dysentery put before us so admirably by Dr Adams. We have been treating dysentery patients at two of our military hospitals from the end of January to the end of September this year when our beds were suddenly required for other reasons and we had to transfer all our patients to the Liverpool School. First we had to consider what we were going to lay down as a standard of reasonable cure. We decided that we should give a course of the pharmaceutical medley of drugs, as already suggested, and then send the patients to a British Red Cross convalescent home for 10 to 14 days, where they would receive a minced diet. They would then return to hospital when their stools were examined again and they were sigmoidoscoped to note the effect of the change from hospital. If the stools were positive they were given a repeat course at above using any injections of 1 grain emetine, euremetine, or emetine bismuth iodide. Again they were sent away and examined on return.

In the unsuccessful cases a course of sulphaguanidine in large doses and if still intractable, a 7-day course of 37 grammes of sulphasuccidine was given. We considered that if the stools remained negative and the sigmoidoscopic appearances were normal and scrapings proved negative after 14 days final stay on ordinary diet at a convalescent hospital, we had obtained a reasonable cure.

Regarding the results of sulphasuccidine, out of fifteen cases treated as above as a final effort there were seven complete failures, one appeared to be cured, while seven were transferred to Liverpool. Yesterday I discovered that of these seven, two had relapsed and five remained under treatment. Sulphasuccidine did not hold out much hope.

Taking this standard of cure, we found that out of ninety-five cases who had received treatment over 9 months only forty (42 per cent.) have returned to duty, twenty have been unabded and twenty seven still in hospital, which means that 49·4 per cent. are still ineffective.

As Colonel HARGREAVES has pointed out, it is most important to eliminate secondary streptococcal infection by penicillin and also to eliminate other thogenic conditions such as bacillary dysentery We have found flagellates ry common indeed, and we have considered it wise to eradicate them before arting the specific treatment While concentrating on amoebic dysentery e must bear in mind other forms of dysentery especially when stools are nsistently negative for *Entamoeba histolytica* Frequently malaria and kala ar may be missed Even carcinoma recti may be overlooked sigmoidoscopic amination may show suggestive ulceration but if a digital examination is rried out the consistency of the tumour mass will become evident.

I stress the psychological atmosphere in the treatment of these cases cheerful ward sister, a general uplift and encouragement are essential. In place can an occupational therapist be better employed than in a dysentery ard this diversional therapy is important in keeping the patients minds easantly occupied during their long courses of treatment When this was troduced into our wards there was an immediate improvement in the general orale

With regard to penicillin therapy I have not had an opportunity of trying As to diodoquin, I have ten cases under treatment at the present moment d the reports are encouraging but they always are with any new drug in e treatment of amoebic dysentery

Another point often missed is that men arrive home emaciated, and in me cases very short of vitamins, in particular the vitamin B complex This is been very marked in some instances and others have shown the symptoms d signs of beriberi

With regard to the particular strains of *E histolytica* I think there must a difference in type because although the drugs we are giving remain the me nevertheless after a man has had large quantities of emetine (in one our series 120 grains emetine hydrochloride) the entamoeba still survives It is also remarkable that after much emetine, or auremetine, etc. a an will suddenly develop an amoebic hepatitis Therefore, it becomes clear at our difficulties are not over and that emetine and its products do not pear to be the whole answer to the treatment of amoebic dysentery I agree th Dr ADAMS when he says that it is important and indeed essential that search should be instituted without delay to produce a drug which will erilize the bowel in the shortest possible time in order to save the patient om a protracted illness and much suffering and also to save the taxpayer e heavy burden of the cost of hospitalization, treatment and finally in all robability a long period of disability pension.

Sir Philip Manson-Bahr said he shared the anxiety of others regarding e lack of response of those virulent war time amoebic infections to the generally cepted treatments He had been impressed by a certain resemblance in hat was now reported to happenings a quarter of a century ago under almost

similar circumstances. It had to be realized that in war time amoebiasis assumed a more complex and virulent aspect than in the more easy-going conditions of peace. It would appear that the clinical severity or otherwise of intestinal amoebiasis depended upon the resistance of the tissues to invasion by *Entamoeba histolytica* and in these virulent toxic cases which have been described he had, moreover to reckon with secondary bacterial infection. But the chief factor in the present situation lay in what he had emphasized for many years—an unreasoning belief in the therapeutic value of unstinted and sometimes entirely uncontrolled emetine injections. No one would belittle the *immediate* effect of hypodermic emetine in the active stage of the disease with vegetative amoebae in the faeces, but in chronic intestinal amoebiasis, where the cystic forms are being passed, emetine, when given by this route, had no effect, for the simple reason that the drug never reaches the pregnant stages. The effect of periodic intensive courses of hypodermic emetine, a practice which appears so dear to the heart of many physicians, was to his mind most harmful in rendering *E. histolytica* emetine fast, so that subsequent treatment by emetine bismuth iodide or other compounds is rendered ineffectual. He had published figures from a large series of cases which he had studied and followed up during the last 20 years to show that this obviously takes place. It was possible, as had been shown, to produce emetine fastness in cultures of *E. histolytica*, and he was convinced that the same process took place with equal facility in the intestinal canal.

It should be hardly necessary for him to stress the supreme importance of treating the patient as well as the disease. These exhausted, debilitated and often ill nourished war casualties required, surely different handling from the average well nourished and otherwise healthy individual who picked up this infection in the course of his daily round. It obviously would be most unwise to fall on those admittedly sick men and deluge them straight away with blunderbuss anti amoebic treatment. Their resistance should first be built up by blood and plasma injections, by glucose and salines, and by adequate nourishment. He had since 1926 been in the habit of treating chronic amoebic dysentery by what is known as the combined method, by the simultaneous exhibition of emetine bismuthous iodide (or some allied compound) with retention enemata of quinoxyl (yatren chiniofon, anayodin).

He did not propose to weary his hearers by details of this method, but he would like to point out that in many of the so-called incurable war infections the minutiae of this treatment had not been observed. Very often it appeared that the emetine bismuth iodide was given in a form (such as keratin-coated tablets) in which it is not absorbed, but is passed unchanged through the intestinal canal. Retention enemata are also given in too big a bulk or injected so quickly that they cannot be retained. These points had been elaborated in a paper he had just completed[*] The speaker did not

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[*] *Lancet*, 1944 **2**, 718.

pretend to have had access to large numbers of amoebic cases during the last five years such as had fallen to the lot of officers in the Services. But from time to time some of the unfortunates discharged from the Army during this r had been referred to him as a last resort. He did not claim to be infallible, but he would quote two examples, one of whom was present at the meeting. In both, success had been obtained by attention to the minutiae of this treat-

One, a soldier from India, had been suffering intermittently from chronic amoebiasis for 13 years and had undergone the whole gamut of anti-amoebic drugs, not once but repeated many times. He had been subjected to three courses of emetine bismuthous iodide, sometimes combined with oxyl enemata, including one at the London Hospital at the end of 1942. In May 1943 he was referred to the speaker who gave him his standard .. taking care to ascertain that the emetine bismuth iodide was actually being absorbed. He had been checked on many occasions since and was now we u and employed on munitions. The second, an officer from Burma, had infected in June 1942, and 3 months afterwards had developed a liver .. ess. He had relapsed many times since and with a recrudescence of his hepatic abscess in April 1943. He was invalided out in January 1944 still ..u numerous *E. histolytica* cysts in the faeces. It seemed probable from his history that the emetine bismuthous iodide had never been adequately absorbed. Under the speaker's direction he underwent a further course in September 1944. He is now well and vigorous. The faeces have been examined on many occasions with a negative result. The cysts disappeared after the second dose of E B I and have not been seen since.

He had recently in these resistant cases been in the habit of presaging the anti amoebic course by protein shock therapy with the idea of desensitizing the patient. He put forward this suggestion for consideration for those who .. confronted by this problem at present.

Finally he was very averse to ringing the changes on all arsenical and other compounds which had been advocated in the past 20 years for the i .. *t of amoebiasis and he was in full agreement that there was ample need for improving the therapeutic treatment of this important disease.

Dr E M Lourie Dr Adams and Colonel Hargreaves are to be congratulated on their clear and concise outlines of the difficulties which they have encountered in the treatment of amoebic dysentery. I would like, further to compliment Dr Adams on all the hard work that lies behind his remarks, for I know from daily association with him, how much industry and devotion are involved. There must be few physicians in England who treat more cases of amoebic dysentery and he, and those who are condemned to be his assistants, seem to spend all their waking hours surrounded by high mountains of those sinister little containers or among the all too many unfortunates whose fate it has been to fill those containers. It is no less than inspiring to watch Dr Adams and his little army of helpers of all ages and all sexes, and

ranging in cultural distinction from an Emeritus Professor down to a lab girl with the school certificate, working their way slowly methodically and relentlessly like so many determined ants, through those great accumulations of excrement

I am somewhat diffident about saying more, for the little that I might contribute to this particular discussion must come as from a theorist with practically no experience of the hard bones of the specific problems involved What I do say must therefore be in all humility whilst in the presence of those who are themselves bearing the heat and burden of the day

It seems to me from Colonel HARGREAVES account, that he really did not give a fair trial to what he called the Liverpool treatment. He treated a number of cases by this method, and an equal number by one of his routine procedures. The "Liverpool lot came out badly but Colonel HARGREAVES admits that there may have been a tendency to give the experimental treatment-course to the more severe cases. One naturally wonders whether the result might not have been significantly different if alternate cases, without any selection, had been treated by the two methods under consideration.

The essential lesson to be gathered from Dr ADAMS contribution is, if I understand it, that emetine alone cannot be depended upon to eradicate an infection, and one must therefore resort to a combination of drugs and it seems to follow then, that such a combination must be used at the very beginning of treatment If deferred until after a series of injections has been given of emetine alone unsupported by any adjuvant, then success will be jeopardized, since the amoebae may well have been started down the slippery slope of acquired drug resistance These are very important conclusions from both the practical and the theoretical viewpoints. They imply clearly that emetine must never be given alone combined treatment, and by intensive dosage, must be instituted at the very start and there is no salvation in any other known course of procedure.

A number of aspects crop up here for the worker in experimental chemotherapy some of which have already been tackled with positive results. For example it was shown by HALAWANI[1] at the Liverpool School, that the acquirement of emetine resistance by Entamoeba histolytica by repeated exposure to the drug is no myth. Apparently it really does happen But we know nothing of the possible dangers lurking behind combined therapy in amoebiasis. Let me quote two examples of the pitfalls of combined therapy in experimental trypanosomiasis. There is the so-called "interference phenomenon" par-fuchsin alone is curative and salvarsan alone is curative. But give the two drugs together under certain conditions, and the one will interfere with the action of the other [a] Then there is the case of treatment by tartar emetic. Administration of this drug alone in subcurative doses does not lead to drug

HALAWANI A   (1930)   Ann trop Med Parasit., 24 773.
[1] BROWNING, C. H. & GULBRANSEN R. (1922)   J Path Bact., 25 385
[a] SCHNITZER, R. (1928).   Z f Immunitätsf 47 116   48, 23   49, 387   49 3rd.

resistance but treat your animals first by an arsenical and then by tartar emetic, and you will very rapidly produce emetic-resistant trypanosomes[4] Nothing is known yet of similar possibilities in amoebiasis and nothing is known the possibility hinted at by Dr Adams that the development of resistance to emetine by the amoebae may automatically involve increased resistance to other amoebicides

The sting of Dr Adams contribution is for me in the tail He is right the experimental chemotherapist has not helped very much Until he does he had better keep off his high horse and content himself with watching from ground level or as he may flatter himself by thinking from the grand stand while the practising physician battles in the arena below

Dr C A Hoare Though more than 70 years have elapsed since *Entamoeba histolytica* was incriminated as the causative organism of amoebic dysentery there remain some serious gaps in our knowledge of the aetiology of amoebiasis. As already mentioned by Dr Adams the effect of amoebic infection in man may vary considerably but we are still in ignorance regarding the respective parts played by the parasite and by the human host in the development of disease

Some of the differences have been attributed to the existence of pathogenic and non pathogenic races or strains in *E histolytica* among which those differing in size should be specially noted In 1917 Wenyon and O Connor, on the one hand and Dobell and Jepps on the other first demonstrated that *E histolytica* comprised several races differing from each other in the mean dimensions of their cysts The question of races has been re investigated more recently by Russian (Gnezdilov 1934 Zertchaninov 1934) and American (Sapero *et al* 1942) workers who have established the existence of two main races (1) a *large one* with cysts having a mean diameter of about 11μ and occurring in 37 per cent of cases and (2) a *small* race with cysts measuring on the average about 7μ and occurring in 56 per cent of cases while the remaining 7 per cent represent mixed infections In practice a diameter of 10μ can be taken as the dividing line between the two races.

The large race corresponds to the conventional *E histolytica* which is pathogenic to man and cats and may produce the well known symptoms of amoebic dysentery while the small race (sometimes described as *E hartmanni*) differs from the large one in certain physiological features thus (1) the small amoebae do not ingest erythrocytes and (2) they do not produce dysenteric symptoms in experimental infections of cats Furthermore there seems to be a general agreement of opinion that in *human* infections neither dysentery nor liver abscess, or any other severe symptoms are ever associated with the presence of amoebae of the small race On this account, it is believed that amoebae of the small race are non pathogenic to man.

[1]Yorke, W., Murgatroyd, F & Hawking, F (1932) *Ann trop Med Parasitol* 26 577

As regards the large race, it varies considerably in pathogenicity its effects ranging from symptomless infection in carriers to typical amoebic dysentery. The causes of this fluctuation are not definitely known but a number of hypotheses have been advanced to explain it.

Most British and American workers are in agreement with WALKER (1913) whose classical experiments have demonstrated that the pathogenicity of *E histolytica* depends rather upon the susceptibility of the human host than upon any difference in the virulence of the parasite strain

Other parasitologists (notably DESCHIENS in France, and WESTPHAL in Germany) hold that *E histolytica* is itself not pathogenic but the symptoms of dysentery are due to its association with certain bacteria present in the intestinal flora These bacteria are said to damage the walls of the gut and thereby prepare the way for the amoebae which can then attack the tissues, producing the characteristic lesions. This view may have some bearing on the cases described by Colonel HARGREAVES and Brigadier PRIEST Finally the foremost French parasitologist, BRUMPT (cf 1906) believes that the large amoebae producing quadrinucleate cysts belong to two distinct species, which are morphologically indistinguishable one *E histolytica* (or *E dysenteriae*) is pathogenic, whereas the other *E disper* is non-pathogenic and responsible for most of the infections in symptomless carriers in non-tropical countries.

While the first two hypotheses appear to be plausible and worthy of further consideration, BRUMPT's views are not accepted by most observers.

From this brief survey it is evident that the position is far from clear and stands in need of further investigation especially as regards the small race. Until further evidence in support of its non pathogenicity is forthcoming, all infections with the small race should be regarded with suspicion, and treated accordingly In the meantime, it would be desirable to keep separate records of the occurrence of the large and small races, and to continue observations and experiments on the effects of infections with the small race of *E histolytica*, until the problem is solved.

Dr H J Smyly I would like to add a few words, from experience in North China at the Peiping Union Medical College and Cheeloo University Medical College, with special reference to three drugs vioform, carbarsone and brucea.

At P U M C we adopted chiniofon in place of E.B.I shortly after its introduction by MÜHLENS and MENK, and it continued to be our standard treatment there and at Cheeloo for many years. For some years at Cheeloo we had a series in course of observation of alternate cases treated with vioform and carbarsone Many of these cases were followed up 2 or 3 months after treatment with faecal examinations. The research was interrupted by war and the results were not collected and analysed, but we gained the impression that both drugs were efficient and about equally so. If cysts were found after a course of 10 days treatment it was repeated with the same drug as we were

working on a comparison of the two In a few cases instead of our usual 10
, course I gave 1 week of each with apparently good results and I think
combined treatment deserves a fuller trial

Following the publication of Dr LIU HSIAO-LIANG of his paper on the
effect on *E histolytica* of the ancient Chinese remedy called ya tan tzu
— the seeds of *Brucia javanica* (or *amarissima* or *sumatrana*) we carried out
extensive trial of this drug This study was also broken off by the war
d most of the records lost, hut an account of the preliminary stage of the
periment is being published Ya tan tzu, to which the PRESIDENT has already
...erred, has unquestionably a specific amoebicidal action hut the crude drug
., in our experience less effective than chiniofon, vioform or carbarsone.

Dr J G Willmore In reply to the PRESIDENT'S question, I tried conessine
several occasions after the last war and found it to be no good.

As to penicillin, I have had the same experience as the earlier speaker It
proves the clinical condition dramatically but does not eliminate the amoebae
tried it in one case of ulcerative colitis without the slightest effect. In this
bowel was swarming with streptococci—which may or may not have been
be cause of the condition, but they were penicillin resistant.

I think it might be a good thing to give a preliminary course of penicillin
all these severe resistant (amoebic) infections as it is possible that penicillin
helps to render the patient more susceptible to standard anti amoebic treatment.

Dr A R D Adams, in reply Much ground has been covered and the
hour is getting late I most heartily agree with SIR PHILIP MANSON BAHR,
onel HARGREAVES and Brigadier PRIEST that a liberal diet and treatment in
genial surroundings are fundamental to progress and the civil hospitals
greatly handicapped in the first respect. I am engaged in a wrangle at
present moment to get these men the rations they have become accustomed
o in military hospitals When they come into civilian hospitals they are reduced
civilian rations and they do not like this I hope my remarks were not taken
i any attack on the Army Medical Services I might say that I have the very
... admiration for their efforts to deal with a variety of situations during
war

Lt -Col E H Vere Hodge and Lt -Col W R M Drew (contribution
o discussion submitted after the meeting).

In conversation, after the discussion on amoebiasis, we both agreed that
more time should have been spent in considering the criteria of cure on com
letion of treatment. The number of patients with typical amoebic dysentery
is small, compared with those suffering from less obvious forms of the disease

Such patients may never have had actual dysentery and, though usually without symptoms are nevertheless in poor health and have subnormal sugar tolerance. The only physical sign likely to be found is tenderness and thickening in the region of the caecum, and possibly iliac colon. After treatment evidence of the disease may even be less obvious, though the patient may remain a source of infection to others.

Not only in these patients but in all cases of amoebiasis we consider the following criteria of cure should be strictly applied —

   (1) General constitutional recovery with return to normal weight.

   (2) On abdominal examination there should be no thickening or tenderness of the colon, especially in the region of the caecum and iliac colon, nor tenderness and enlargement of the liver

   (3) On stool examination vegetative and cystic forms of *Entamoeba histolytica* should be absent in at least ten specimens   This test alone may be fallacious when cysts are passed intermittently as is often the case

   (4) On sigmoidoscopic examination active ulceration should be absent   During the recovery period flecks of stool and mucus may be seen adhering to the mucosa, the result of colonic dysfunction.

   Sometimes even when the mucous membrane of the colon appears intact cysts continue to be passed in the stools   In these cases, radiological examination with a double contrast barium-air enema is of value to demonstrate ulceration out of reach of the sigmoidoscope.

It follows therefore, that all the above points must be taken into consideration when assessing cure and, even when positive signs have disappeared, it is desirable in chronic cases, to refer the patient to a convalescent home for a month, and at the end of this time examine him again before returning him to duty

Finally   mention should have been made of those cases in which the appendix appears to be the nidus of infection and in which appendicectomy is necessary in addition to full medical treatment, before cure can be effected

COMMUNICATIONS

TROPICAL ULCER

BY

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Tropical ulcer is a rapidly spreading ulcer occurring usually on the
ower extremities of the body which quickly assumes a phagedaenic character
d is accompanied by considerable pain local oedema sloughing and a sero-
sanguinous discharge. The edges of the ulcer are undermined and the margins
c considerably raised.   (Roy 1938) This is the definition of the *British
ncyclopaedia of Medical Practice* and can hardly be improved.

The condition known as tropical ulcer is not just caviare to the general
nblic, nor is it of academic interest only as a glance at the figures in Appendix
on page 268 will show   In an Editorial the *Lancet* (1943a) stated —

the true tropical phagedaenic ulcers of unknown aetiology are
constant problems.

More recently the *Lancet* (1943b) states —

" Tropical ulcers which mostly affect the legs and are an important
cause of disability among native workers have been much improved by
penicillin, although the supply is too limited for this treatment to be used
extensively

In 1832 E. C. Smith wrote —

" Ulcus tropicum, or tropical sloughing phagedaena, constitutes an important economic problem in Nigeria, as in other colonies Though it occurs usually in isolated cases the condition may assume epidemic proportions particularly in labour camps. With few exceptions the ulcers involve the lower limbs and a history of trauma is obtained in practically every case The term tropical sloughing phagedaena is an apt one as in the majority of these cases the ulcers are covered with a foul purulent or necrotic layer which, on being cleared away leaves a raw granulating base surrounded by a raised, sometimes rolled, indurated edge   In later cases the ulceration may extend deeply and expose muscle tendon and bone. As regards diagnosis, it may be stated as a general rule that any ulcer in the smears from which fusiform bacilli or spirochaetes of the Vincent s type or both, are found, is a tropical ulcer

In the *Annual Report of the Calcutta School of Tropical Medicine, 1932,* it is stated —

" Phagedaemic ulcers, Naga sore frontier sore, occurring on the legs of coolies working on the tea plantations during hot weather usually following trauma. The discharge from these sores is infective and inoculation experiments made on the arm of subjects produced a vesicle which formed into a shallow ulcer and rapidly healed in 6 or 10 days. On the other hand, inoculations made into the foot gave rise to a typical Naga sore which persisted for a considerable time. The infectious discharge was filtered through Chamberland L3 and L5 filters, but the filtrate was not infectious. Smears showed the fusiform bacillus, most commonly and staphylococci, streptococci and diphtheroids on one occasion Vincent s spirochaetes were seen."

Early in December 1942, the Pathologist was asked by the Chief Medical Officer (Dr H Jamison) of the Anglo-Iranian Oil Company to investigate and treat an unstated number of cases of tropical ulcer in Persian labour   Some of these cases had been previously treated by the Consulting Surgeon and the Consulting Physician, and a few had been invalided as incurable.

The conditions under which the investigations and treatment were to take place were (1) all cases were treated as out patients beds not available (2) no additions to the diets of the patients treated was possible; (3) the only dressings and drugs available in unlimited quantities were plaster bandages, sterile vaseline and sterile gauze. The help of the Persian doctors and nursing staff and dressers in the out patients department was allowed and was invaluable great credit is due for their enthusiastic assistance Many of them had not seen a tropical ulcer heal until they worked with us. The Shipping Manager very kindly arranged for a supply of whale oil for the clinical trials this was most gratefully received.

Any consideration of the aetiology of tropical ulcer must include a study of (1) the seed and (2) the soil.

1 THE SEED

The factor of infection is of fundamental importance The responsible organism is not a virus but is a common infective bacterium found in dirt. A dirty skin is an essential factor in causation (DENNIS personal communication 1943) and the flora of a dirty skin includes staphylococci streptococci diphtheroid bacilli Vincent s bacilli and spirochaetes At one time or another all these organisms are found in the discharges from a tropical ulcer but the most difficult to dislodge and the organism most frequently associated with relapses and delayed healing is Vincent s bacillus. Once this bacillus ceases to appear in serial smears of wound discharge it is generally safe to say that healing will follow quickly Tropical ulcers when first seen are usually already infected with multiple microbic strains Further cross-infection is to be avoided despite the dictum of TRUETA (1939) this problem is reviewed at some length by MILES (1941) The condition known as wound phagedaena (CALLASI and DUFF 1941) synergistic infective gangrene of skin (MELENEY 1933) progressive streptococcal ulceration (LANGSTON 1938) spreading subcutaneous or cutaneous gangrene (MITCHINER and COWELL, 1939) is similar in many respects to tropical ulcer Streptococci Gram-positive diplococci and *Bacillus proteus* are found in this condition the streptococcus—which is often anaerobic and usually non haemolytic—is usually chosen as the pathogenic scapegoat, and MELENEY recommends the application, to the lesion, of a special preparation of zinc peroxide In spite of the obvious similarity it is considered that a typical tropical ulcer differs completely from MELENEY's ulcer but the latter kind of ulcer must always be borne in mind and anaerobic cultures made of the pus if there is the slightest doubt.

In spite of the reluctance of nearly all workers on the problem of tropical ulcer to incriminate Vincent s bacillus and spirochaete as the infective agents most workers admit the presence of these organisms in the majority of the ulcers they have studied. One author—the late E C SMITH—even goes to the length of stating that the presence of fusiform bacilli or spirochaetes in the discharge of an ulcer is a determining factor in the diagnosis In conditions such as Vincent s angina and trench mouth authors do not hesitate to apportion the blame It is true that not a few of these ulcers seem to be free from Vincent s organisms when first examined such ulcers however are usually of long standing and some previous therapeutic application may have banished the fuso-spirillary group It is also true that some ulcers will heal in spite of the continued presence of Vincent s organisms—a hard fact not easy to explain. It is possible that, as in the case of Japanese, extermination is a necessary preliminary to defeat for these persistent little invaders again on the same analogy the organisms may throw up the sponge unexpectedly

It is certain that—when the process of healing is followed closely by serial smears—the disappearance of Vincent s organisms is the forerunner of victory and usually presages complete and speedy healing The reappearance of

Vincent's organisms in an ulcer temporarily freed is a bad sign and means considerable prolongation of treatment. In our opinion Vincent's are the predominating organisms but infection in the form of a tropical ulcer will not occur unless two other essential factors are present dirt and trauma.

The story of zinc peroxide is taken a stage further in an Editorial in the *Lancet* (1942). Many of the papers of F. L. MELENEY are quoted, the Meleney ulcer is superficial spreading gangrene involving only the skin and due to the presence of an anaerobic non-haemolytic streptococcus combined with a staphylococcus both organisms have to be present together The special preparation of zinc peroxide is marketed by only one manufacturer in America it is effective against haemolytic and non haemolytic streptococci, gas gangrene organisms, anaerobic cocci Gram-negative bacilli " fæcospirochaetal abscesses soon lose their foul smell "

HOFFMAN (1941) combined zinc peroxide iodoform, sulphanilamide powder equal parts with liquid paraffin he called this " Zisp " and found it very effective in the treatment of air raid wounds.

2. THE SOIL.

The factor of dirt was mentioned above other predisposing causes are trauma, malnutrition, associated diseases such as malaria helminthiasis bilharziasis, guinea worm infection, etc stagnation of circulation in the dependent limb anatomical deficiency of smaller arterioles in the tissues between the ankle and knee and autophytic influences—many patients are disappointed when the lesion is healed.

(*a*) *Dirt* This is an essential factor Professor E. W DENNIS tells us that European soldiers, in splendid health and enjoying a good quality and ample diet, developed tropical ulcers at the sites of minor trauma after they had been involved in heavy and continuous fighting for days or weeks and so had been unable to clean or otherwise take care of themselves mental and physical exhaustion were also factors in these cases. A dirty skin may be less resistant to infection than a clean skin, and a dirty skin carries in itself the seeds of infection. Tropical ulcer in well fed Europeans is mentioned also by Mayer-BAHR (1935).

(*b*) *Trauma*. This is the other essential factor even very slight trauma is sufficient. Mere rubbing of the skin under a plaster bandage often starts a new tropical ulcer near one that is already under treatment, infection is no doubt helped by the presence of discharges from the original ulcer Most authors agree that the discharges from a tropical ulcer will readily produce a fresh lesion if inoculated into healthy skin (PATTERSON 1908 Short 1932, etc). Even in dirty people tropical ulcers are rare if their feet and legs are protected by good boots and puttees or similar coverings.

(*c*) *Malnutrition* There is little doubt that malnutrition is an important factor it was a prominent one in nearly all our cases. In our cases the cost

standing dietary deficiencies were protein calcium, vitamins A, B and C These are the significant deficiencies mentioned by CORKILL (1939)

We were unable to correct the diet of our patients and it is interesting that given suitable treatment—most of our cases with the exception of one individual healed, even though slowly in spite of their dietetic errors. It is likely that if we had been able to make up the deficiencies in the diets of our patients their ulcers would have healed much more rapidly

Marked loss of body weight—previous to treatment—in patients with wounds is given malign prognostic significance by STUDLEY (1936) and PAYNE 1941)

(d) *Associated Diseases*   Great weight is attached to this factor by VIGORS EARLE (1942) and it is an important cause of debility and lowered resistance We agree that associated diseases should be diagnosed and treated, but we must emphasize that—even if the associated diseases are neglected—the ulcer will heal with proper treatment

Proper treatment of associated diseases will here again encourage rapid healing of the ulcer

(e) *Stagnation of Circulation*   This point was emphasized by DICKSON-WRIGHT (1930) in his work on varicose ulcers in Europeans.   Some tropical ulcers have a varicose complication but it is not common   Nevertheless, the principles brought out by DICKSON-WRIGHT are equally important in the treatment of tropical ulcers.   (This is dealt with in detail under the heading of   The Lock-Up Treatment of Tropical Ulcers,   p 264)

EARLE mentions the anatomical peculiarities of the vascular system in the ulcer area   and gives two references to important original work.

In causation the factor of stagnation is, without doubt, important   in treatment, however it is *essential* to neutralize this factor

In our cases cold weather and rain seemed to favour the occurrence of tropical ulcer   in India wet warm weather is the season of high incidence There is no doubt that cold extremities favour the factor of stagnation.

(f) *Autophytic Influences*   These are hardly mentioned in the literature, but among our patients were very important indeed, and non recognition of this factor is the cause of many failures in treatment.   Autophytic influences are almost ruled out by our plaster method, hence probably the large number of defaulters we have to record

(g) *Tissue Inertia*   A very common condition was indolence of the ulcer after prolonged, and apparently successful, treatment.   The ulcer would improve slightly and remain stationary   This complication is not a simple and has a nutrition component, an infection component, a mechanical component and a " tissue growth factor   component.

This cause of delay was also eventually overcome but not until investigation and the trial of remedies—some vaunted, others devised by had shown the way   This problem also is discussed in more detail

Epitome of aetiology   Filth   Food   Friction   Fuso-spiril

(HUNT 1941). Age, state of nutrition, protein intake, antecedent loss of serum vitamin balance, state of general circulation and blood are points made by WHIPPLE (1940). Mortality after operations for peptic ulceration is directly proportioned to antecedent loss of weight (STUDLEY 1936). The question of hospital dietary is very important. Adequate quantities of nicotinic acid in the diet have been an unattainable ideal for many cases of fuso-spirillosis.

<center>PRESENT INVESTIGATION</center>

In the investigation now to be described, a record was kept of the name, identification numbers, occupation lesions treatment, daily food, body physique organisms in the smear of discharges and history of trauma for each patient. Generally patients were seen once a week or when progressing well once a fortnight. A few patients were alarmed by the discharge which oozed through the plaster and was sometimes very smelly. Such men came up at all times and were reassured and told to report again on the day appointed.

Small quantities of the following ointments and drugs were obtained mainly through unofficial sources, and were tried on eighty-five selected cases it was usually easy to decide whether the mixture was effective or not after only a few applications —

(1)	Ointment A	5 per cent. sulphathiazole in vaseline with urea 1 per cent. all sterilized.
(2)	Ointment B	Bismuth iodoform paraffin paste. (Bipp of RUTHERFORD MORISON)
(3)	Ointment C	40 per cent whale oil and 60 per cent. vaseline, sterilized. (VIGORS EARLE.)
(4)	Ointment D	Sterile vaseline. (W H OGILVIE.)
(5)	Ointment E	Vaseline with 1 per cent. urea, sterile.
(6)	Ointment F	Zinc oxide, iodoform, liquid paraffin. (Zipp of CONNELL & BUCHANAN.)
(7)	Ointment G	Olive oil 30 per cent. vaseline 70 per cent. sterile.
(8.)	Ointment H	Linseed oil 30 per cent. vaseline 70 per cent. sterile
(9)	Ointment I	Para-chloro-meta xylenol zinc oxide liquid paraffin.

The following   washes   were tried —

Wash 1	2 per cent. copper sulphate in water
Wash 2	2 per cent. silver nitrate in water
Wash 3	Trichlorophenylmethhiodosalicyl.
Wash 4	N N Dichlorozodicarbonamidine (" Azochloramid) 1 in 500 in triacetin
Wash 5	Sterile physiological saline.

We intended to try the following powders but two of them proved to be obtainable —

(1.) Powder a   Proflavine powder (MITCHELL and BUTTLE, 1942)— ~~~ble.

(2.) Powder b   Special zinc peroxide (MELENEY 1933)—unobtainable.

(3.) Powder c.   Potassium permanganate (available in small quantities)

The information obtained from our cases has been expressed in the form ~ tables (Appendices B and C) *

On the appointed days every patient was questioned.   Then the ulcer was examined and a smear of the discharge made.   The ulcer was roughly ~~, washed or powdered—sometimes both—and one layer of sterile medicated with A, B or C etc. cut roughly to the shape and size of the applied with sterile forceps   then a layer of one thickness of sterile ~auze, impregnated with sterile vaseline was applied over the top of the medi ~ed layer   The vaselined gauze was big enough to cover the ulcer completely ~th at least 4 inches overlap in all directions   One plaster bandage, width inches was next applied and the patient told the date of his next appointment

The medicated powder was applied with a home made flour dredger holes in the   rose   very small   only a very thin covering of powder one grain thick   was necessary in most cases

The   healed   group can be classified as follows —

~eeks of healing	1	2	3	4	5	6	7	9	10	11	13	14	15	18	20	21	22	27
~ of cases	1	12	8	9	8	4	6	2	2	1	1	1	1	1	1	1	1	1

As will be seen, the average case took just over 2 weeks to heal—actually ~/27 weeks, and the majority were healed in less than 7 weeks.

The rate of healing varied roughly with the size of the ulcer but the appli ~ of correctly chosen dressings was a very big factor in rapid healing

For instance, Case 7 did not heal for 12 weeks during which time a variety medicated applications were tried   then, after another short period of ~aling his ulcer relapsed.   At this stage permanganate powder was applied ~ice and subsequent healing was rapid and complete.   There was, of course, sutophytic element in this case, but the powder was too strong even for ~phytism

It is unnecessary to describe the progress of the cases in any detail, a brief ~udy of our records showed clearly the factors that influenced healing   It enough to say that the   lock up   method is clearly vindicated and the use f bipp or zipp very strongly indicated with permanganate powder as a tower f strength when obstinate infection had to be treated.   *The effective control f infection was vital to healing the more effective the control the more rapid the* ~   There were very few exceptions to this rule

Another interesting case was Case 73   He had had applications of tartar

* Through lack of space Appendices B and C are not included in this paper   but ~ey have been filed at Manson House for reference if desired.—ED

emetic elsewhere and a dry red scar had formed over his large ulcer. The tissues had been so depressed by this application—which had, however, abolished all Vincent's organisms—that many weeks were occupied in coaxing the granulation tissue and epithelium to grow normally again.

It is generally stated that encasing in plaster encourages the growth of granulations but depresses epithelium—in our experience—always very vaseline and ointment—epithelium did not seem to be retarded in growth at all, just the opposite in fact. We ascribe this stimulation of epithelial growth to (1) effective control of infection (2) protection of the very delicate surface of the ulcer from even very slight mechanical disturbances (3) the pansement spécifique " (4) avoidance of drying

In addition to the size of ulcer and choice of dressings, one other factor appeared in the cases that took longest to heal, this was position other things being equal, an ulcer on the ankle or foot took much longer to heal than one on the knee. The anatomical peculiarities of the ulcer area " described by Vigors Earle (loc. cit.) may have influenced this development.

Twenty six patients " defaulted " before healing appeared to be complete. Of these at least nine are known to have healed, but did not report again because they thought they did not require any more treatment. All the defaulted " cases would have healed if they had persisted in their treatment.

In two of the defaulters " (Cases 1 and 9) a definite autophytic element was observed. These patients reacted badly to any sign of improvement in the ulcer and were apprehensive of complete healing.

Many of the patients did not like the plaster dressings and demanded a daily dressing they liked to be able to take the dressing off if the wound ached, smarted or was painful—healing seemed to be a secondary consideration to these individuals.

APPENDIX A.—MONTHLY CASE INCIDENCE IN 1943-1942.

1943	Number of cases.	1942	Number of cases.
		December	218
		November	305
		October	246
September	665	September	81
August	654	August	40
July	284	July	23
June	144	June	23
May	166	May	28
April	75	April	8
March	130	March	5
February	74	February	13
January	138	January	33

No case is reported as "healed" unless the ulcer area was completely covered by healthy dark skin with a firm dry scar in the centre. It was found that if patients were not followed up to this stage the ulcer tended to relapse; none of our healed cases relapsed.

The ulcer—however large and long in healing—did not appear to produce any immunity in the patient.

SUMMARY

The aetiology of tropical ulcer is discussed: the basic facts can be epitomized in the alliterative mnemonic,

Filth, Food, Friction   Fuso-Spirillosis

The treatment of these ulcers is discussed, special emphasis being laid on the 'lock up' method. The ointments bipp and zipp were found very effective, but powdering the ulcer with a thin layer of crystals of potassium permanganate—as a preliminary to the dressing—produced rapid healing in some otherwise resistant cases.

Of eighty five cases, fifty-nine were completely healed in an average time of just over 2 weeks: the majority healed in less than 7 weeks: the most obstinate case took 27 weeks to heal. Twenty six cases defaulted, but none of these are known to have healed. Autophytism was an important element in causing defaulting.

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SURVIVAL OF TRANSFUSED RED CELLS IN BLACKWATER FEVER CIRCULATION AND OF BLACKWATER RED CELLS IN NORMAL CIRCULATION

(PRELIMINARY REPORT)

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INTRODUCTION

The sudden haemolyses that occur in blackwater fever have been attributed to a number of different causes. Some have suggested that there is a haemolytic strain of malaria that is responsible, others that there are specific parasites present that give rise to the condition, and yet others that there are circulating haemolysins.

The fact that in some haemolytic conditions such as haemolytic jaundice, defective red cells have been shown to be present has led some to suggest that in blackwater fever there is also some abnormality in the erythrocyte that renders it peculiarly liable to destruction.

It has already been shown that neither haemolytic strains of malaria nor specific parasites can account for the destruction of red cells that occurs in blackwater fever (FOY and KONDI 1936) It was later shown that normal red cells transfused into a rapidly haemolyzing case of blackwater fever from several different compatible donors were destroyed in the blackwater circulation just as were the patient s own cells   It was concluded from this that it is not the erythrocytes themselves that are defective in this disease but that red cell destruction is due to other causes, perhaps circulating haemolysins (FOY, KONDI and MOUNJIDIS, 1941).

The sudden forced removal from our laboratories in Salonika as a result of the war prevented us from following up this work with more extended observations.   Later however thanks to the courtesy of the Public Health Department of Portuguese East Africa, we have been able to follow up and elaborate the work using a modification of the technique of ASHBY (1919) and MOLLISON and YOUNG (1941 1942) which we have adapted to meet the special conditions found in blackwater fever with its profound and recurring haemolyses

Our problem was rather more complicated than that of MOLLISON and YOUNG or of DACIE (1941) since we had to deal with repeated haemolyses and multiple transfusions occurring in the space of a few hours or days. In consequence the transfused " O " cells were constantly disappearing and had to be replenished by further transfusions.

The questions that we set ourselves to answer were (1) Are the transfused cells destroyed during the haemolyses as are the patient's own cells ? (2) Has the transfused cells a normal survival time in the blackwater circulation once the haemolytic crises have ceased ? (3) Have blackwater fever cells, taken during and after haemolytic crises, and transfused into normal or malarious individuals, a normal survival time ? (4) Is blackwater plasma haemolytic when transfused into a normal or malarious individual ?

DACIE and MOLLISON (1943) have shown that red blood cells transfused from normal donors into haemolytic jaundice have a normal survival time and that red cells transfused from haemolytic jaundice into normal individuals have a shortened survival time.

DACIE has concluded from this that the basic abnormality in haemolytic jaundice is in the corpuscles and that extra-corpuscular factors are not likely to play an important role   The experiments reported below tend to show that in blackwater fever at least, the fundamental factor is extra-cellular and that haemolysae normal cells in the blackwater fever circulation as well as bring about changes in the blackwater fever red cells that render them susceptible to destruction even in normal circulations   Thus the situation in haemolytic jaundice and blackwater fever would appear to differ in that whatever brings about the lysis of the red cells in haemolytic jaundice is not capable of affecting normal red cells transfused into patients with active haemolytic jaundice.

It should be noted that in conditions like blackwater fever where liver function is apt to be grossly disturbed, and vomiting present, the varying states of hydration and dehydration will bring about differences in the blood count that may be independent of blood destruction. Serial blood counts as and conditions are not necessarily a true index of blood destruction, a fact brought out by variations in haematocrit readings and blood volumes as determined by Evans Blue.   For these reasons we consider that quantitative estimation of the pigments in blood plasma and urine as well as blood counts, are a surer guide to what is taking place than red cell counts alone.

A further point of interest has arisen during the course of this study, namely the greater frequency of transfusion reactions in blackwater fever when compared with other conditions. Accesses of haemolyses and haemoglobinuria are fairly common after transfusions in blackwater fever and this has no doubt led to the view expressed by some that blood transfusion is contra-indicated in blackwater fever   In the present state of our knowledge it is difficult to say whether the exacerbations of haemolysis and haemoglobinuria that sometimes follow a transfusion are really due to the transfusion or merely to the general

of the disease since it is known that in blackwater fever the haemolyses occur
in waves, a patient may pass black urine for a number of days and then pass
clear urine for some time to be followed by another bout of haemoglobinuria,
and there may be several such clearings and exacerbations before the patient
finally recovers (FOY and LEWIS 1941) It is therefore very hard to decide
whether an access of haemoglobinuria that follows a transfusion is due to the
transfusion or to a normal wave of haemolysis characteristic of the disease

The frequency of auto-agglutination in blackwater fever as in other severe
anaemias, makes it desirable to transfuse such cases with low titre homologous
blood and not to rely on compatible universal donors

Further the use of cell suspensions is recommended rather than whole
blood, so as to reduce the amount of high titre agglutinins that might be present
in large volumes of fluid. (LOUTIT, 1943)

METHODS

The method employed for estimating the survival time of the cells trans-
fused into the cases of blackwater fever was that of differential agglutination
and was based on the simple principle of taking an A " or B group black-
water fever and transfusing with blood from a compatible O group donor,
removing blood from the vein, adding anti- A or anti- B serum to agglu-
tinate the patient's own A or B cells and then counting the remaining
unagglutinated O cells.

There are a number of factors that contribute to the survival of transfused
red cells such as the age of the blood, the amount and type of anti-coagulant
used, etc., all of which have been fully investigated by MOLLISON and YOUNG
(1940) and BUSBY et al (1940) Further the method of differential agglutina-
tion involves an error of some ± 10 per cent. When, however all these factors
have been taken into consideration there seems to be no question that the
method can yield valuable information concerning red cell survival in various
haemolytic conditions

Since there will always be a certain number of red cells in any individual
that are not agglutinated by specific anti serum, it follows that the number
of such cells must be determined before transfusing with group O cells
otherwise it would obviously be impossible to distinguish the patient's own few
unagglutinated cells from the unagglutinated transfused O cells In practice
therefore, one determines the number of say unagglutinated A in an A
group individual before giving the transfusion with O blood, and subtracts
the number so obtained from the total number of unagglutinated cells found
after the transfusion in order to find the actual number of O cells present
in the A individual after the transfusion.

In estimating the number of unagglutinated cells present, we have counted
all the large squares in the Burker chamber and divided the total number of

cells so found by the number of squares counted. The figures in the dire represent the average number of cells in one large Bürker square. If it is desired to express the results in cells per cubic millimetre the numbers given in the charts have simply to be multiplied by 25 000 according to the formula

$$\frac{\text{Number of cells counted} \times 100 \times 250}{\text{Number of squares counted.}} = \text{number per c.mm.}$$

This differs somewhat from the calculation of MOLLISON who represents the highest number of unagglutinated cells present after the transfusion as 100 per cent. and the lower numbers as varying proportions of this percentage. This method was not practicable for our material since we had to deal with constantly recurring haemolyses and repeated transfusions.

For estimating the survival times of blackwater fever red cells that were transfused into normal or malarious individuals, group O" cases of blackwater fever were used as donors and normal or malarious group "A" individuals as recipients and the survival times of the transfused "O" cells estimated by the method outlined above. As a check on our technique, different agglutination tests were performed on a normal healthy group "A" individual transfused with blood from the same group O" donor as was used in the transfusion of Case 1 blackwater fever (Experiment 7).

We were careful to use anti-serum from the same batch for all the tests done on the same individual so as to compensate for any unavoidable difference in the titre of the anti-sera. All anti-sera were stored at 4 C. until required and each ampoule contained only sufficient for the day's tests so that no aged anti-serum was ever used. The anti-sera used in this work were prepared for us by Dr E. GAYNOR LEWIS, of the South African Institute for Medical Research to whom we are also indebted for much helpful advice.

Blood for the transfusions was taken into sodium citrate in normal saline so as to give a final dilution of 0.38 per cent. In nearly all the cases the blood was used immediately and in no case did more than 15 hours elapse between bleeding the donor and giving the transfusion and during this period the blood was kept at 4 C. so that storage deterioration was never a factor in our work. Direct compatibility tests were carried out in all cases. Blood samples for the agglutination tests were taken 24 hours after the transfusion and thereafter according to the haemolytic crises.

The blood was collected from the same vein into tubes containing 0" c.c. of 2 per cent. ammonium and potassium oxalate for each 5 c.c. of blood. Counts were all done in standardized pipettes and chamber and the same pipette was always used for the same individual. In the case of the total count, 1,880 cells were counted so as to standardize the error at ± 3 per cent (PONDER, 1930). Blood was never taken up beyond the mark and then drawn back, as this has been shown to introduce considerable errors. Absolutely dry pipettes and chambers were of course always used.

Haemoglobin was estimated by the acid haematin method of Newcomer
in a Klett biocolorimeter or spectrophotometrically on a Pulfrich photometer
Plasma oxyhaemoglobin and methaemalbumin were estimated quanti
tively using the extinction coefficients recently found by Fox and OTTENBERG
1941) The pigments were identified by means of the Hartridge reversion
spectroscope.

Price Jones curves were drawn on a Leitz Panphot apparatus from slides
stained for 1 minute in Leishman and 1 minute in aqueous eosin thus standard
zing any shrinkage of the cells that may have taken place during staining
(NDER, 1934.)

Reticulocyte counts were done by the wet method after 15 minutes
incubation at 37° C

Haematocrit was estimated by the Van Allen method and bilirubin by
e original method of van den Bergh since haemoglobin was present in the
lasma.

MATERIAL.

The cases were typical blackwater fever which is very common in Portu
uese East Africa. All the cases were hospitalized and under our complete
control and the laboratory examinations were done by us in the Anti Malaria
tation Lourenço Marques where the cases occurred.

Experiment 1

Male, aged 35 years European, with a history of three previous attacks of black-
ater fever the last one in March, 1943   On 30.8.43 the patient took 25 cg
quinine at 9 a.m. presumably for malaria and half an hour later passed a quantity of
ark urine He continued to pass similar urine throughout the day

On 31.8.43 he entered hospital. The urine on his entrance was black and contained
yhaemoglobin, methaemoglobin and urobilinogen. The patient was very icteric,
miting slightly and had a spleen II (Hackett) Red cell count was 1 685 000 A full
ood examination done on 1.9.43 showed the following —

lethaemalbumin	= 230 mg	Schumm s test	= + + + +
B C.	= 1 160 000 p.er c.mm.	Reticulocytes	= 0 2 per cent.
b	= 3-4 grammes per cent.	Malaria parasites	= Negative
aematocrit	= 10·0	Unagglutinated	
aemobilirubin	= 8·0 mg		red cells = 2 2 = 55 000 per c.mm.
xyhaemoglobin	= 270 mg		

Blood and urine examinations were done each day and the results are given in Table I
d Chart 1 which also show the variations in the total red cell count compared with
ne rises and falls in the unagglutinated cells during the course of the illness and for
days after the last transfusion of O blood.

For the first 5 days of the illness the patient was given 1 gramme of oral quinine
lphate   On 6 10 43 Plasmodium vivax rings appeared in the blood and 1 gramme of
urine daily was given orally for 5 days without any untoward effects

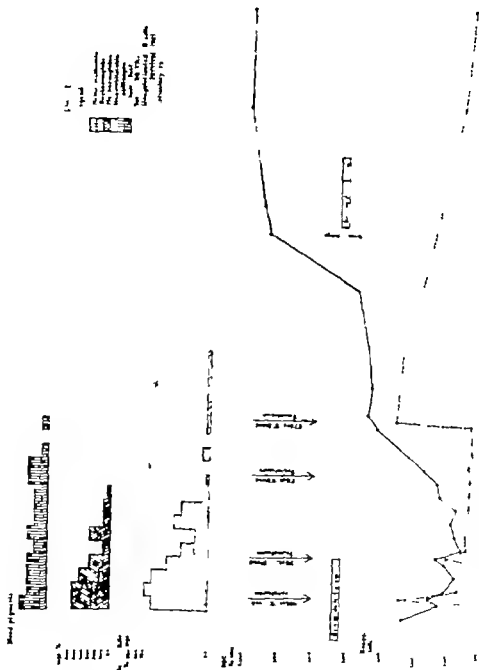Transfusions as will be seen from Chart 1 were given on 1.9 43 4 9 43 10.9 43
nd 14.9 43.

A transfusion of 400 c.c of compatible O blood was given on 1.9 43 A blood
count taken 4 hours after this showed that the red cells had risen to 1,300 000 and the
umber of unagglutinated cells to 12 3 per large Bürker square (= 307 500 per c.mm.)

TABLE I

Date	Mils R.U.C. per c.mm	Hb. grammes per cent.	Haema-tocrit per cent.	Retics. per cent.	Schaum Test	Plasma Pigments mg per cent.			Urine Pigments			No. Unaught treated Cells per 1 Large Bürker Square	No. Unaught treated Cells per mm
						Met Hb.	Oxy Hb.	Bili-rubin	Met Hb.	Oxy Hb.	Uro-bilinogen		
30.8.43 31.8.43	1,843	3.1	10.0	0.2	± +				+ −	+ + + + + +	Pos.		53,000
1.9.43 100 c.c. trans. "O" blood	1,100				−	90	170	5 mg	+	+ or −		2.2	207,500
4 hrs after trans.	1,200											12.3	
2.9.43 3.9.43	1,000 900	3.3 3.1	10.0 9.5	0.2 1.2	+ + −	190 390	290 150	9 mg 4 mg	+ −	+ or − + +		2.7 2.5	92,500 87,500
4.9.43 320 c.c. trans. O blood	1,000	3.3	10.0	3.0	+ + + + + +	90	100	8 mg	+	+		1.5	37,500
4 hrs after trans.	1,300	4.0	11.5			Neg.			Neg.	+ +		8.1	125,000
5.8.43 6.9.43 7.9.43 8.9.43 9.9.43 10.9.43	813 817 993 995 1,164 1,290	2.8 2.9 3.0 3.1 3.3	8.5 9.4 10.6 10.0 12+	8.0 11.0 21.0 25.0 7.0	+ + + + + + + + + + + +	100 100 100 80 Neg.	4 mg. 2 mg 3 mg 4 mg 1 mg n.a mg		+ +	− − − + +		2.3 2.8 2.8 9.1 1.0	67,500 68,500 61,000 60,000 50,000 60,000

110.43	3 740			45
71043	4 100			28
141043	3 090	9.9	35.0	15
111143	5 410	13.5	45.0	

				107 000
				70 000
				37 500

TABLE II

Date	Milla R.B.C per c.mm	Hb. grammes per cent	Haematocrit per cent	Retics per cent	Schumm's Test	Plasma Pigments mg per cent			Urine Pigments			No Unagglutinated Cells per 1 Large Burker Square	No Unagglutinated Cells per c.mm
						Met HAB	Oxy Hb	Bili rubin	Met Hb	Oxy Hb	Urobilinogen		
29.9.43	3 000 (V)	—	—	—	—	100	150	—	++	++++	++++	—	—
31.9.43	1 799 (V)	3.9	12.0	0	++	60	40	1	Neg	Neg	++++	2.0	65 000
22.9.43	1 430 (V)	—	—	—	—	0	130	—	++	++	++++	—	—
140 c.c trans "O" blood													
22.9.43	1 170 (V)	3.5	12.0	3.4	— +	Neg.	10	8	—	+	+++	13.0	3 5 000
24.9.43	1 451 (V)	—	—	6.6	±		Neg	—	—	+	+++	—	—
26.9.43	1 528 (V)	4.1	—	13.8	Neg					+	+++	0.1	227 000
27.9.43	1 03* (V)	6.6	16.5	10.0				0.7	—	Neg	+++	7.0	100 000
.8.9.43	2 148 (F)	7.4		7.9				0.8			—	7.0	100 000
.9.9.43	0.9 (F)			11.0							—	5.5	135 000
1.10.43	2 793 (F)	0		13.0							—	2.0	7 000
4.10.43	3 173 (V)	10.3	33.0	8.0							—	0.0	15 000
12.10.43	3 890 (V)	11.5	36.0	4.0							—		
18.10.43	4 880 (V)			0.0							—		
10.11.43	4 710 (V)	11.5	38.0	—	—	—	—	—	—	—	—		

Chart 1

During the succeeding days the red cell count as well as the number of non agglutinated cells fell rapidly and blood pigments were present in the plasma and urine as additional evidence of blood destruction as will be seen from Chart 1 The second transfusion of " O " blood given on 4.9 43 was followed by a rise in both red cells and unagglutinated cells.

During the next 24 hours there was a rapid fall in the patient s red count to 812 000 and a fall in the unagglutinated cells to 2 3 (57 500 per c.mm.) Thereafter the blood count rose and the plasma and urine cleared of pigments

As a check on our agglutination method, 270 c.c. of group A blood was given on 10 9 43 This resulted in a rise of the red cells but there was no increase in the number of unagglutinated cells as was to be expected from a transfusion of A blood

On 14.9 43 a fourth and final transfusion of 270 c.c. of group O blood was given resulting in a sharp rise in the unagglutinated cells to 13-4 (33o 000 per c.mm.) Six days after the transfusion there were 12 8 cells (332,000 per c.mm.) Reference to the graph in Chart 1 will show that there was a steady fall in the number of unagglutinated cells during the following days and that 30 days after the transfusion there were 2 8 (70 000 per c.mm.) unagglutinated cells remaining An agglutination test done 56 days after the transfusion showed that all the transfused cells had disappeared It is clear from this that normal cells transfused into a case of blackwater fever 9 days after the cessation of the haemolytic process have a survival time of about 30 to 35 days whilst those transfused during the haemolysis were destroyed as were the patient's own cells.

Experiment 2.

A child aged 11 years, European, born in the colony and living in highly malarious region with a history of malaria since a baby of 6 months Two other individuals in the same house also had blackwater fever During September the child had repeated attacks of malaria for which he was given sporadic doses of quinine with temporary improvements in his health

On 18.9 43 the child appeared well on 19 9 43 he had a rigor in the morning and was given 25 cg of quinine at 9 a.m. after which he appeared well He was sent, however to Lourenço Marques by car a distance of 250 km. and during the journey vomited and passed black urine. He was immediately sent to hospital The child seen on the 20th, was very icteric but did not appear very toxic Spleen II his blood count was 2,600 000 and he was passing adequate quantities of black urine containing oxy haemoglobin, methaemoglobin and urobilinogen.

A full blood examination done on 21 9 43 was as follows :—

R.B C	= 1 780 000 per c.mm.	Oxyhaemoglobin	= 40 mg per cent.	
Hb	= 3 8 grammes per cent.	Schumm s test	= + +	
Haematocrit	= 12·0 "	Reticulocytes	= 0 2	
Haemobilirubin	= 12·0 mg	Malaria parasites	= Negative	
Methaemalbumin	= 60 mg	Unagglutinated cells	= 2·6 = 65 000 per c.mm.	

The rest of the patient's history is shown in Chart 2, p 280 and Table II p 277

The case was given only one transfusion of 140 c.c. of group O blood on 22.9 43 Sometime during the next 24 hours there was a small haemolysis as shown by the reappearance of oxyhaemoglobin in the urine and this brought the count down to a figure lower than it was before the transfusion The unagglutinated cells rose from 2·6 before the transfusion to 13·0 (32o 000 per c.mm.) afterwards. Thereafter as will be seen from the chart, the red count slowly rose and the unagglutinated cells gradually fell. The interpretation of this case is somewhat difficult since the fall in the blood count after the transfusion actually reduced the red cells from 1 430 000 to 1 120 000 although the unagglutinated cells reached 13·0 (325 000 per c.mm.) We can only suppose in the absence of evidence to the contrary that if the haemolysis had not followed the transfusion the unagglutinated cells would have been very much more than 13·0 (325 000 per c.mm.) Reference to the graph in Chart 2 shows that during the following days the unagglutinated cells fell gradually so that 5 days after the transfusion there were only 9 1 (227 000 per c.mm.) and 29 days after all the transfused cells had disappeared, thus giving the same picture as in Experiment 1

CHART 2.

CASE 2.

Experiment 3

In order to ascertain whether erythrocytes from a haemolyzing case of blackwater fever have a normal survival time when transfused into normal individuals a group O case of blackwater fever was bled during the haemolytic crisis while the plasma contained large amounts of oxyhaemoglobin and methaemalbumin. The blood so obtained was allowed to sediment in the ice chest for 12 hours and 100 c.c. of the red cells so obtained transfused into a normal healthy person. As will be seen from Chart 3 the number of unagglutinated red cells before the transfusion was 2 8 (70 000 per c.mm.) Twenty four hours after the transfusion the number had risen to 9·6 (360 000 per c.mm.) During the following 7 days the number fell to 2 2 (55 000 per c.mm.) thus indicating that black water fever cells taken during the haemolytic process and transfused into normal circulations are destroyed very rapidly This work is being continued and will be reported fully later

Experiment 4

To discover whether cells taken after the haemolytic process had stopped had a similarly short survival time, blood was taken from the same group O " case as in Experiment 3 but 10 days after all signs of haemolysis had disappeared, and transfused into a normal individual. The patient from whom the blood was taken developed another access of haemoglobinuria 5 days after we had taken the blood from him, so that the short survival time found in this case may be due either to changes that took place in the red cells as a result of the previous haemolysis, or to changes that were taking place preparatory to the haemolysis that occurred 5 days after we had taken the blood The details of the experiment are shown in Chart 3 Before the transfusion the recipient had 3 2 (90 000 unagglutinated cells per c.mm.) 24 hours after the transfusion of 70 c.c. of cells, this number had risen to 8·0 (200 000 per c.mm.) During the next 5 days the number fell to 4·0 (100 000 per c.mm.)—again illustrating the same shortened life span as seen in Experiment 3 This work is also being continued with larger amounts of red cells

Experiment 5

In order to ascertain whether blackwater fever circulations can haemolyze normal red cells transfused into them long after the haemolysis has stopped, a group A " case that had his last attack of blackwater 18 months previously was transfused with blood from a group O donor Before the transfusion, the number of unagglutinated cells present in the recipient was 1 2 (30 000 per c.mm.) 500 c.c. of group O blood was given and the number of unagglutinated cells rose to 22·0 (550 000 per c.mm.) After 60 days, there still remained 3·6 (90 000 per c.mm.) after 75 days there were 1·4 (35 000 per c.mm.)—thus showing that after a sufficient period of time has elapsed from the onset of the blackwater the viability of the red cells becomes more or less normal.
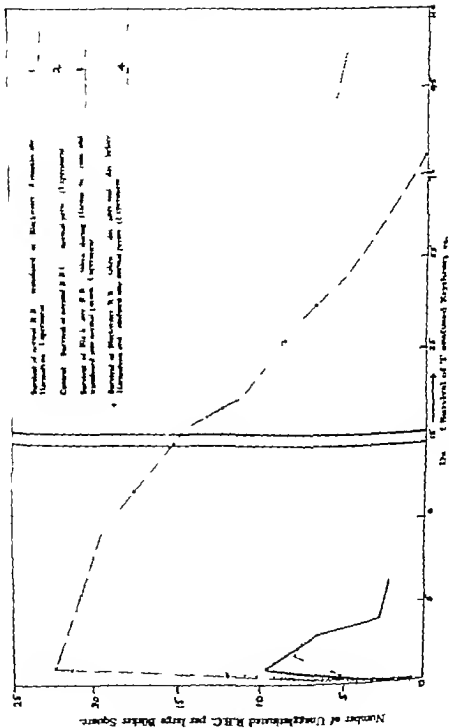
Experiment 6

At this stage it seemed of interest to discover whether blackwater fever plasma when injected into malarious individuals had any haemolytic effects 500 c.c. of plasma was therefore taken from a case of blackwater during the haemolytic crisis, when the plasma contained 225 mg per cent. haemoglobin and 365 mg per cent. methaemalbumin and transfused into an individual undergoing an acute attack of malaria with fever 40° C and intense rigor and with blood containing falciparum rings + + + + Immediately after the transfusion 2 grammes of quinine were given thus apparently reproducing all the conditions that are generally regarded as preceding an attack of blackwater fever Blood was taken from the patient half an hour after the transfusion and again after 4 hours Apart from a slight increase in the indirect van den Bergh, there was no other untoward feature the urine remained clear throughout the whole period of observation and the patient made an uneventful recovery

Experiment 7   Control

As a check on our methods, a differential agglutination test was carried out on a normal healthy group A individual using the same group O " blood that was used

CHART 1.

the blackwater fever Experiment 1   Before the transfusion the number of unagglu
tinated cells was 4 3 (107 500 per c.mm.)   24 hours after the transfusion it was 13·0
(325 000 per c.mm.)   23 days after the transfusion there were 10·0 (250 000 cells per
c.mm.)   75 days after the transfusion he had 6 7 (167 500 per c.mm.)   103 days after
the transfusion there were 4 8 (120 000 per c.mm.)   It is clear from this that the red
cells had a much longer survival time in a normal individual than in the blackwater fever
one, and that the survival time as indicated from this case is normal.   Details of this case
given in Chart 3

DISCUSSION

We believe that insufficient attention has been paid to the resemblances
and differences between blackwater fever and haemolytic jaundice and feel that
a consideration of the haemolytic processes that occur in these two conditions
ill do something to clarify the fundamental problem of red cell destruction
·n these and other conditions.

From the experiments carried out above it seems clear that red cells trans
d into haemolytic cases of blackwater fever are destroyed in the patient s
circulation just as are his own cells, and that once the haemolyses have stopped
the survival time of the transfused red cells increases as the length of the con
valescence.   For example in Experiment 1 red cells transfused 9 days after
haemolysis had ceased had a survival time of some 30 to 35 days   Experi
ment 2 gave the same survival time of 30 days   In Experiment 5 red cells
transfused into a case that had had his last attack of blackwater fever 18 months
previous to our transfusion, had a survival time of some 75 days   It seems
there is some factor in the blackwater fever circulation that has the power
·f destroying all red cells that come into contact with it irrespective of their
origin and the influence of this factor diminishes as the convalescence proceeds.
The fact, however that red cells taken from cases of blackwater fever during
and before the haemolytic crisis and transfused into normal persons are also
destroyed very rapidly (6 days) indicates that there is also some defect in the
·~ cell itself that renders it peculiarly susceptible to destruction even in normal
circulations.   (Experiments 3 and 4)   We believe however that the primary
factor at work in blackwater fever is extra-corpuscular and that it can bring
about changes in all cells that are brought into contact with it and render them
susceptible to destruction

It has been shown that the circulation of haemolytic jaundice has no such
pc ~ to destroy normal cells transfused into it, although haemolytic jaundice
cells transfused into normal individuals have a shortened life   In this respect
there is a fundamental difference between the situation in blackwater fever
and haemolytic jaundice.   This may mean no more than that in the latter disease
where cell destruction is never so pronounced as in blackwater fever there
only sufficient haemolysin available to destroy a minimal number of cells. The

characteristic spherocytosis $\left(\text{M C T} = 3 \cdot 0 \dfrac{\text{M C.D}}{\text{M C T}} = 1 \cdot 95 \quad 1 \cdot 0 \right)$ and altered

osmotic saline fragility in haemolytic jaundice, neither of which is [...] in blackwater fever

$$\left(M C T = 2 \cdot 4 \frac{M.C.D}{M.C.T} = 2 \cdot 9 \quad 1 \cdot 0 \right)$$

make it appear th[...]

neither of these changes is really fundamentally associated with the [...] to haemolysis, a point borne out by the fact that removal of the spleen in haemolytic jaundice leaves the spherocytosis and altered resistance to [...] unchanged. Further the characteristic morphology and behaviour of the [...] cells in haemolytic jaundice indicate that the major defect in this disease [...] be in the cell itself whilst in blackwater fever they would appear to be [...] not only in the cells but also in their environment, the latter factor being [...]

The nature of the factors that are responsible for destroying normal [...] cells transfused into blackwater fever circulations and bringing about ch[...] in the blackwater fever erythrocytes that render them susceptible to haemolysis even in normal circulations is quite unknown. MAEGRAITH, FINDLAY and MARTIN (1943) have suggested that the haemolysin in blackwater fever may be due to a reduction in the inhibitory factors generally present in tissue [...] and sera, and that the balance between lytic agent and inhibitor is shifted to the lytic side. It is too early to assess the importance of these observations at the present moment. HILL and CASTLE (1940) have stated that in such conditions as icterus gravis neonatorum, haemolytic jaundice, etc., intravascular lysis followed by spherocytosis and changes in osmotic fragility are more likely to be factors in bringing about blood destruction than are circulating haemolysins. In the case of icterus neonatorum it is now known that Rh factors are responsible. In haemolytic jaundice DACIE's work would appear to show that stasis is not likely to be a factor unless, as he says, there is selective action on the discoidal erythrocytes.

DACIE believes that in haemolytic jaundice the basic abnormality lies in the red cells in blackwater fever it seems that there is some abnormality in the red cells but there is as well some more fundamental extra-cellular factor that has the power to haemolyse normal cells that come into contact with it.

It may be argued that, since normal cells are destroyed in blackwater circulations and blackwater fever cells in normal circulations, the basic defect in this disease is not in the cells but that there is some mechanism at work that brings about changes in all red cells that come into contact with it, which is different from the situation in haemolytic jaundice where normal cells are not affected. This mechanism may be stasis in the spleen since most authors agree that congestion is the most characteristic feature of the circulation in this organ.

Whether during this stagnation in the spleen the red cells come under the influence of lyso-lecithin as has been postulated by some authors (BERGENHEM and FAHRAEUS [1936] FAHRAEUS [1939] FAHRAEUS [1941]) is difficult to say at the present state of our knowledge. Such a view might link up with the fact that the red cells of blackwater fever and haemolytic jaundice have reduced

resistance to lyso-lecithin (FOY and KONDI, 1943) We have stated elsewhere (loc. cit.) that this altered resistance to lyso-lecithin in blackwater fever does indicate that there is some defect in the red cell in this disease which is not manifested by any change in saline fragility The exact interpretation of this altered lyso-lecithin fragility is not understood but it may indicate that changes have taken place in the lipo-protein complex of the cell walls that renders the cells more susceptible to destruction We have dealt fully with this question in a previous paper (FOY and KONDI 1943)

The explanation of the absence of effects from the transfusion of black-water fever plasma taken during the height of a haemolytic crisis and transfused into a patient with an acute attack of malaria may mean nothing more than that ¯ ᵗ plasma was used or that any haemolysins that were present were ... up in bringing about the haemolysis in the blackwater fever patient. It is interesting to note however, that although the plasma failed to produce any sign of haemolysis in the recipient, nevertheless red cells transfused into the blackwater case immediately we had taken blood from him, were haemo-lyzed, indicating that there were haemolysins present at the time we took our blood. No doubt variations in the amount and type of blood pigment in the donor's plasma will influence its effect on the recipient.

From these experiments and from DACIE's on haemolytic jaundice, it would seem that the haemolytic processes in blackwater fever and haemolytic jaundice are fundamentally different although both are to be regarded as intra-... haemolyses as can be shown by the presence of methaemalbumin /or a positive Schumm's test in both diseases

SUMMARY

1 Red cells transfused from compatible group O donors into haemo-lyzing cases of blackwater fever were destroyed in the blackwater fever circulation ... as were the patient's own cells The survival time of normal red cells into cases of blackwater fever 9 days after the haemolyses had ... was 30 to 35 days after 18 months the survival time of transfused red cells was normal. This indicates that there is some factor present that ... all red cells that come into contact with it, and that the influence this factor diminishes as convalescence lengthens.

2. Red cells taken from a haemolyzing group O case of blackwater ...ver and transfused into a normal group A person had a survival time of only 6 days, indicating that there is some defect in the blackwater fever red cells that renders them more susceptible to destruction even in normal circulations.

3 Five hundred c.c. of plasma taken from a haemolyzing case of black fever laden with oxyhaemoglobin and methaemalbumin and transfused into a patient with an acute attack of malaria failed to produce any sign of haemolysis, other than a slight rise in the indirect van den Bergh no doubt associated with the pigments present in the plasma used. This suggests that

D

either insufficient plasma was used or that any haemolysins present had already been used up in the blackwater fever case.

4   Normal red cells transfused into haemolytic jaundice have a normal survival time but haemolytic jaundice cells transfused into normal circulation have a shortened survival time, indicating that in this disease the primary defect is in the red cells, a fact borne out by the marked spherocytosis and altered resistance to saline, neither of which is present in blackwater fever.

5   The fact that the more severe haemolysis in blackwater fever is not associated with changes in cell morphology or altered saline fragility suggests that neither of these changes may be really fundamentally connected with red cell destruction, a point borne out by the failure of splenectomy to alter the spherocytosis or saline fragility in haemolytic jaundice, although it stops the periodic haemolytic crises.

6   All these facts suggest that the haemolytic processes in blackwater fever and haemolytic jaundice may be of a fundamentally different nature that in blackwater fever there appear to be factors operating that can bring about changes in any red cells that come into contact with them which is not the case in haemolytic jaundice

7   Lyso-lecithin fragility is increased in both blackwater fever and haemolytic jaundice making it appear that this may be a better guide to haemolytic tendencies than is saline fragility or spherocytosis. Whether this lyso-lecithin fragility is in any way connected with splenic stasis or changes in the lipoprotein complex of the cell membrane is at present impossible to say

8   On account of varying states of hydration due to kidney upsets and vomiting serial blood counts alone are not necessarily a true index of blood destruction in blackwater fever

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CLINICAL FILARIASIS DUE TO
ACANTHOCHEILONEMA (FILARIA) PERSTANS

BY

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Blood infection with larvae of *Acanthocheilonema perstans* is quite common in certain parts of Africa, but it rarely causes clinical symptoms. From the cases here described it would appear that in certain circumstances it may give rise to quite serious clinical disturbances.

Over a period of 4 months in which these cases were observed, routine blood examinations revealed the presence of *A perstans* larvae in two other people who had no symptoms which could be ascribed to filariasis.

Both cases were Hausa natives of Sokoto Province, Northern Nigeria.

CASE 1

D H. an African female aged about 35 a welfare nurse was admitted to hospital 11 4 43 complaining of upper abdominal pain, diarrhoea and vomiting After admission her diarrhoea ceased, though for the 1st day she vomited on several occasions copious quantities of a mahogany-coloured fluid.

On examination there was deep tenderness in the epigastric area and the liver was enlarged to four fingers breadth below the costal margin. No jaundice was apparent and no muscular rigidity A stomach tube was passed and 17 ounces of fluid aspirated.

Stool. Red blood cells slight cellular exudate a few cysts of *Entamoeba histolytica* and some hookworm ova.

Urine Reaction highly acid. Sp gr., 1020 No abnormal constituents

Blood   R.B.C.s, 3 600 000   Hb 65 per cent.   C.I., 0·9   W.B.C.s, 8,500   polymorphs 61 per cent.   eosinophils, 2 3 per cent.   No parasites seen.   Kahn negative.

In view of the tender liver and its enlargement a course of emetine was started.   On the 2nd day the pain had not improved though the general condition was quite good.

In the early morning of the 3rd day she became suddenly worse, the pain was severe and the patient very shocked with cold clammy extremities and weak radial pulse   On examination the liver tenderness had increased, there was some guarding in the upper abdomen and percussion in that region was dull. Deep respirations were painful.   In the chest there were no pulmonary signs, the heart normal apart from very weak sounds.   Pulse 110   Temperature subnormal

Attempts were made to aspirate pus from a possible liver abscess.   Only blood was withdrawn and this was found to contain an excess of polymorphonuclear leucocytes and numerous larvae of *Acanthocheilonema perstans* Owing to the rapid deterioration in the patient a condition a laparotomy under high spinal (percaine) anaesthesia was performed after premedication with morphine and coramine 2 c.c. half hourly   The liver was found to be very congested and enlarged, but there was no evidence of a localised liver abscess Further attempts to draw pus were not successful.   The stomach was distended and there were old adhesions between the quadrate lobe and the lesser omentum The falciform ligament was oedematous and inflamed.   The other viscera were normal.

The patient improved slowly but owing to vomiting the emetine was discontinued after 8 grains had been given.   A recurrence of the pain on the 8th day with a rise of temperature was successfully treated with sulphapyridine At no time were microfilariae seen in the peripheral blood.

Blood (28 4 43)   R.B.C.s, 3,800 000   Hb 65 per cent.   C.I. 0·8   W.B.C.s 7 600   polymorphs, 58 per cent.   eosinophils, 2 1 per cent.   No parasites.

The presence of polymorphs in the blood from the liver suggested suppurative hepatitis, but the temperature was low for this at all times and there was no leucocytosis.   The patient was discharged 6.5 43 with no further symptoms.

CASE 2.

M. N., an African male, aged about 45   a native administration official attended as an out-patient 7 7 43   complaining of upper abdominal pain.   He had had several attacks before at frequent intervals.   On examination there was vague tenderness in the upper abdomen chiefly on the right side.   This was his only complaint and there was no history of dyspepsia.

He was put on a belladonna and bismuth mixture and a diet suggested but the pain became somewhat worse.

On 15 7 43 he was admitted to hospital for investigation as he still had
the epigastric tenderness   The appetite was good at all times   Lungs and
heart were normal.

Blood   R B C.s 4,200 000   Hb., 75 per cent   C I   0 9   W B C.s
9 600   polymorphs 64 per cent.   eosinophils 4 9 per cent.

One larva seen in a careful examination of one thin film and five in one
thick film.   Kahn negative

Stool   Some hookworm ova only   *Urine*   Normal

Though sometimes nauseated from the pain   he never vomited and had no
diarrhoea. Blood pressure, 130/95   The fundi were normal. No evidence of
arteriosclerosis. Nervous system apparently quite normal. Apart from the
pain he seemed to be in good health.

Calling to mind Case 1 a small quantity of blood was aspirated from the
liver with a spinal needle   It was found to contain large numbers of larvae of
A. perstans —

19 7 43   36 per 16 c.mm.   10 a m.   40 per 16 c mm.   12 noon   40 per
16 c.mm. 6 p m.

N B —This was determined by the use of a Thoma counting slide under
the low power

Antiphlogistine plasters relieved the epigastric discomfort. Intravenous
injections of anthiomaline were given, starting on 20 7 43 with 0 5 c.c and
increasing this by 0 5 c.c. every other day until 20 c c had been given
Maximum single dose 2 c.c.

A marked diminution in the numbers of microfilariae in blood from the
liver occurred.

	Blood from the liver	Blood from lobe of ear
24 7 43	*4 per 16 c.mm.	Larvae present.
26 7 43	—	Larvae present
28 7 43	Some larvae seen.	No larvae seen.
2 8 43	Some larvae seen	No larvae seen.

The patient was discharged 12 8 43 after 10 days complete freedom from
pain.

It was possible to follow up this case and there had been no recurrence
of the pain up to 18 11 43

COMMENTS.

1 Both cases were Kahn negative   In one the most marked clinical
finding was a tender enlargement of the liver and the other epigastric pain

and tenderness probably hepatic in origin. The usual causes of painful hepatomegaly—cholangitis, pylephlebitis, suppurative hepatitis, biliary obstruction appeared to be ruled out. Heart and lungs had no apparent lesion. Both cases were acute illnesses from which recovery seemed complete. Granted that if the microfilariae are not directly responsible for the lesions in the hepatic region, is it not reasonable to assume that a heavy hepatic infection with the parasite may not predispose to a bacterial inflammation in much the same way as an injection of quinine may be responsible for a "fixation abscess?

Boukoutcov (1937) reports a case of acute hepatitis in a native soldier which proved fatal in 2 days. He had violent colic and bilious vomiting of sudden onset following a meal. This case was associated with larvae of *A. perstans* in the blood and in sections of the liver microfilariae were seen. He was of the view that the hepatitis was septicaemic in origin and that the parasite played a non pathogenic role.

Moiier (1939) considers that *A. perstans* is not a harmless parasite as high fever has occurred in cases of infestation with the parasite when other causes appear to have been ruled out.

2. In both cases a moderate infestation with hookworm was present but such a large proportion of the population are so affected and it appears to cause no bad effects apart from the moderate anaemia.

3. Although antimony has not the reputation of being much use in the treatment of filariasis, in Case 2 marked diminution in the numbers of parasites occurred after the use of anthomaline and the symptoms were relieved. It must be remembered that the numbers of larvae in the peripheral blood vary spontaneously. Moiier claims that intravenous methylene blue, 1 per cent, 2 to 10 c.c., causes the larvae to disappear

4. Manson Bahr (1940) states that larvae of *A. perstans* are rarely seen in large numbers in the peripheral blood, but are confined to the heart and large vessels, though rarely in the liver. It will be noticed that in these two cases the larvae were present in the liver in large numbers.

SUMMARY

Two cases of severe upper abdominal pain are described associated with a marked infestation of the liver with larvae of *Acanthocheilonema perstans*, suggestive that this filarial worm can give rise directly or indirectly to morbid clinical symptoms.

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DIVIDING FORMS OF *PLASMODIUM FALCIPARUM* IN THE PERIPHERAL BLOOD OF AFRICANS *

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The schizogony of *Plasmodium falciparum* remains somewhat of a mystery particularly with regard to the reasons for its taking place normally not in the peripheral blood, but within certain internal organs   There has however been one feature of this process which appeared to be established beyond doubt   this was that the presence of even early segmenting forms   still more of schizonts in the peripheral blood was an index of the gravity of the infection.   It is accepted that such forms indicate the probability   if not the actual presence of a malarial attack of the   cerebral   type   We believe that dividing forms have never hitherto been found in the peripheral blood of an immune person.

This communication describes the appearance of schizonts of *P. falciparum* in two severe cases of malaria in semi immune Africans and in one immune African who recovered without any specific treatment.

CASE HISTORIES

Case 8941

A semi immune African of the Nyika tribe from Southern Tanganyika His home is in malarious country   in which, however   the annual malaria season probably lasts for a few months only

He had been in Nairobi district for some months prior to admission to hospital on 25.5 43 with a severe attack of malaria which had commenced that day   On admission he was drowsy and confused.   His temperature was

101 5° F and rose on the following 2 days to 105° F   Spleen palpable (one finger breadth) and tender   He had presumably been given some quinine before admission, since his urine gave a positive Tanret reaction on the 3rd day   He was given 0 2 gramme mepacrine on the 3rd, 4th and 5th days. His temperature became normal on the 7th day and he had an unstemptod convalescence   On the 3rd day many (approximately 12,000 per c.mm.) parasites of *P. falciparum* were found including dividing forms, and a few mature schizonts   These latter were not again found but a few asexual parasites were found up to the 8th day

Case 12247

A semi immune African of the kisii tribe from Western Kenya. His home was in an area in which malaria transmission is only present for a short annual season and infections are therefore acquired too infrequently for a substantial degree of immunity to result   He had been in the Nairobi area for a few days, having been previously for some weeks in a highly endemic area at Maseno in Western Kenya. Admitted to hospital on 10.8.43, 3 days after the onset of his attack, slightly confused and showing evidence of acute malnutrition.   His temperature was 103 5° F., he was slightly jaundiced, and the spleen was not palpable.   The C.S.F. was clear with a cell count of less than 1 per c.mm.   On the second day after admission he was put on 10 grains of quinine and 0 1 gramme mepacrine, on the 3rd day 10 grains quinine and 0·2 gramme mepacrine, on the 4th and 5th days 0·2 and 0·1 gramme mepacrine.   His temperature rose to 102 2° on the 3rd day but became normal on the 5th day and he had an uninterrupted convalescence   His urine was negative to Tanret's reagent the day after admission, but contained much albumin and some bile pigment.   On admission many parasites of *P. falciparum* were found and 2 days after this dividing forms were found in small numbers.

Dividing forms were found on the 2 succeeding days (i.e., up to the 5th day by which time the temperature was normal), and gametocytes 2 days later. Asexual forms disappeared on the 7th day and remained absent until discharge on the 11th day

Case 6519

An immune African of the Jita tribe in Northern Tanganyika, close to or on Lake Victoria.   He had been on leave to his home and had returned, travelling through unfamiliar malarious country 8 days before admission to hospital.   He was admitted on 8.4 43 5 days after the onset of an attack of high fever with shivering   Previous to admission he was complaining of his chest, and it is believed that he was given four tablets of sulphapyridine   His urine was negative to Tanret s reagent on the 8th day after admission.   On admission he was moderately ill with a temperature of 101   it rose again on the 3rd and 4th day to 104 5 and 104 (with rigor) but

dropped to normal on the 5th day and remained there. The spleen was enlarged to one finger breadth below the costal margin. On admission he had been selected as one of a control series in some therapeutic trials that were in progress in this hospital. Accordingly he was given aspirin (20 grains daily) only, and received no specific treatment while in hospital, so long as his fever lasted nor for 12 days afterwards. A heavy infection of *P. falciparum* was found on the 1st day of admission. On the 3rd, 4th and 5th days considerable numbers of dividing forms including mature schizonts were found in the peripheral blood. These were not found after the 6th day. On the 4th and 5th days the

parasite count was approximately 27,500 and 10,000 per c.mm. but after this the number of asexual parasites dropped rapidly to nil on the 7th day and except for their appearance in small numbers on the 11th and 12th days, were absent until discharge on the 20th day.

Gametocytes were found from the 8th till the 17th day, after which a 3-day course of pamaquin was given. He was then discharged having had a normal temperature (apart from one rise to 99°) for 15 days. The course of his infection is shown in the accompanying chart.

From his history this man must be regarded as an immune African as his home is in hyperendemic country. But he had been in an area which is

only slightly malarious for the 2 years up to his going on leave. The present attack was more severe than typical attacks in immune Africans.

Appearance of Parasites.

There were no differences between the appearances in the parasites seen in these three cases. In each there were the solid trophozoites of *P. falciparum*, characteristic in size, pigment and appearance. The schizonts contained 12 to 24 merozoites with the typical clumped pigment. Parasitized red cells were not enlarged and in many cases were stippled with Maurer's dots. There can be no doubt of the species diagnosis.

Discussion

There is every probability that if the first two cases had not been treated they would have become dangerously ill, following the anticipated course of events when schizogony of *P. falciparum* is found in the peripheral blood. Indeed, both cases were already showing mild mental confusion when treatment started, and the general picture corresponded with the more severe type of acute malaria as seen in the non immune African. Yet cases moderate and dosage with mepacrine (plus, in one case, 20 grains of quinine) resulted in recovery as rapid and complete as we are accustomed to expect in Africans with malaria of the same apparent clinical severity and similarly treated. Again, it is remarkable that in one case (Case 12247) dividing forms were still present in the blood the day after the temperature had fallen to normal, yet instead of the supervention of a medical emergency convalescence had started.

It seems reasonable to conclude, therefore, that in the case of these two semi-immune Africans the appearance of *P. falciparum* schizonts in the peripheral blood had not quite the serious significance it is generally supposed to have.

The third case (Case 6519) was still more remarkable. From experience of many thousands of other cases of malaria in Africans, both immune and non immune this man was, in our opinion only moderately ill. He was certainly never dangerously ill. That he should have developed an attack of even this severity is probably explained by his slight exposure, or non-exposure, to infection for 2 years, followed by exposure to a strain of *Plasmodium* which he had not previously encountered. Nevertheless he had retained sufficient resistance to enable him to overcome his infection unaided. His case gives still stronger support to the conclusion advanced above.

We are unable to put forward any definite explanation for the appearance of schizonts in these three cases. It is clear from their histories that the appearance of schizonts in the peripheral blood did not indicate an overwhelming infection (*i.e.* a complete breakdown of resistance). No morphological feature of the parasites concerned suggests that they were other than the usual *P. falciparum*, and we see no reason to attribute unusual toxicity to them.

The expulsion of schizonts into the peripheral blood may have been a purely mechanical event, perhaps due to splenic contraction on arrival in a place of high altitude (nearly 6 000 ft) and of comparatively low temperature But we have observed no such event in many similar cases

The possibility of an explanation in terms of tolerance on the part of the immune host seems to be excluded by the similarity of the reaction in the semi immunes.

It may be that a parasite variant is in question and a possible explanation is that certain genetically older strains of *P falciparum* though morphologically typical, are developing habits of asexual reproduction more nearly resembling those of the other species of *Plasmodium*

On the other hand it must be recognized that in the African, who is subject to so many endemic diseases the reticulo-endothelium is already overworked and partially blocked. Under these circumstances the saturated reticulo-endothelium may permit schizonts to escape into the peripheral blood, in the case of even a moderate infection

Whatever the true explanation may be these cases clearly provide notable exceptions to the accepted view that peripheral schizogony in malignant tertian infections in Africans is of the most serious significance.

SUMMARY

1 Three cases of malaria in Africans are described, in which schizonts of *P falciparum* were found in the peripheral blood.

2 Two cases recovered with minimal treatment, and the third on no treatment at all. None of the cases was dangerously ill.

3 It is suggested that there may be a race or strain of *P falciparum* in which peripheral schizogony may occur or that when the reticulo-endothelium is overloaded from any cause an overflow of schizonts may take place without the grave implications which are usually accepted as being associated with such an occurrence.

MASSIVE DOSE TRYPARSAMIDE BY INTRAVENOUS DRIP METHOD IN THE TREATMENT OF TRYPANOSOMIASIS

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The encouraging results obtained by various writers in the treatment of early syphilis by massive dose intravenous drip arsenotherapy prompted the writer to treat cases of trypanosomiasis similarly using the pentavalent arsenical tryparsamide.

The majority of cases infected with *Trypanosoma gambiense* who attend the Colonial Hospital at Tamale are villagers. Their homes may be situated up to 30 miles away from the hospital. In the Gold Coast it is generally considered that a minimum of 12 weekly injections of tryparsamide is essential to effect a cure. In considering these two factors of distance and length of treatment it was not surprising to find in 1943 that over 50 per cent. of trypanosomiasis cases which began treatment at this hospital, never completed treatment. Possibly many of these inadequately treated cases became arsenic resistant.

Forty two consecutive cases of trypanosomiasis in varying stages of the disease were admitted to this hospital and treated by this method. Many of them were also suffering from helminthiasis avitaminosis and chronic malaria.

The objects in view were   (a) To sterilize rapidly the glands and blood of trypanosomes (b) To prevent the development of an arsenic resistant strain of trypanosome (c) To effect cure if possible.

DIAGNOSIS.

The blood and spinal fluid of all the cases were examined on admission to hospital. Gland puncture (G.P.) and examination of the gland juice was carried out in those cases showing enlargement of the posterior cervical glands. Accurate estimation of spinal fluid protein was not possible in the earlier cases owing to lack of facilities. Later C.S.F. protein was estimated by a Sicard Cantaloube rhachialbuminimeter. Spinal fluid cell counts were made in all cases and examination of the deposit after centrifuging for trypanosomes was carried out, both before and after treatment.

TABLE I.

G.P. alone	Blood alone	C.S.F. alone	C.S.F. + G.P. + Blood −	C.S.F. + G.P. + Blood +	G.P. + Blood + C.S.F. −
8	14	14	1	5	1

Positive C.S.F. means that there was either direct or indirect evidence of central nervous system involvement. Thus thirty three cases, i.e. 78 per cent. showed involvement of the central nervous system.

The total number of cases treated was 42. the total mortality seven cases (16·6 per cent.)

TABLE II.

AGE INCIDENCE AND DEATHS DURING TREATMENT

Age in years	1–5	6–10	11–15	16–20	21–30	31–40	41 and over
Cases	—	8	14	6	11	8	5
Deaths	—	—	—	1	2	1	4

Sex incidence. Females, 11. Males, 31.

The primitive conditions which exist in tropical villages result in premature senility. The normal degeneration is hastened by chronic infection with helminths, malaria, filariasis, an ill-balanced and often inadequate diet. Thus an African villager aged 40 years or more, has become more senile than his urban brother. It will be seen in Table II that the mortality was highest in the age group 41 and over.

APPARATUS

(a) A 2-pint glass douche can to serve as the tryparsamide solution container (b) Suitable lengths of rubber tubing (c) An interceptor was improvised by insertion of the base of an intravenous needle into the lumen of the rubber tube, which was then slipped over one end of a glass male urethral irrigation nozzle (d) A screw clamp above the interceptor controlled the rate of flow The apparatus was assembled as shown in the illustration.

Technique

The drug used was tryparsamide (May & Baker) An adult between 0 and 50 kg in weight was given about 2 grammes daily for 6 to 9 days. It
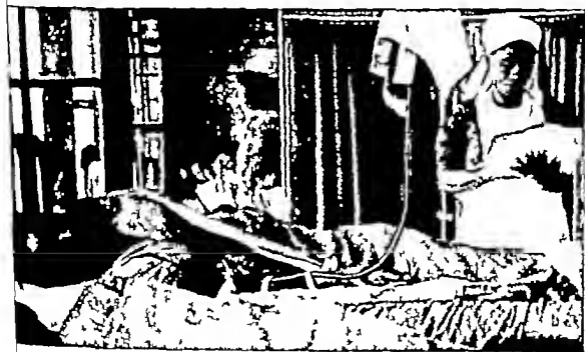
Massive Dose Tryparsamide by Intravenous Drip Method.

was found advisable to rest the patient in many instances for a day after 3 or days treatment, as the high temperatures resultant upon treatment caused varying degree of exhaustion.

The requisite dose of tryparsamide was dissolved in 2 pints of sterile double distilled water in the container which with the other apparatus used had been flushed through with sterile distilled water after sterilization by boiling The patient's arm was immobilized on a splint. A suitable vein in the forearm was entered by a hypodermic needle, which was then connected to the apparatus and the solution allowed to drip Care must be taken to prevent the solution running into the subcutaneous tissues. A rate of about

40 drops a minute allowed 2 pints of solution to flow in about 8 hours. The rate was adhered to as nearly as possible in all cases. A sedative, e.g. dial veronal, was given at the beginning of each day's treatment. Alternate arms were used on alternate days. A sterile towel covered the mouth of the glass container to prevent air borne contamination of the solution.

Length of treatment in hospital

Treatment was usually commenced on the day of admission. Cases were discharged 24 to 48 hours after the completion of treatment. In most instances cases were given 6 days treatment on 6 consecutive days. The more recent cases were given 8 to 9 days treatment with a day's rest halfway through the course. It is not known what the total requirement of tryparsamide is to effect cure by this method. Probably the requirement varies from case to case, depending upon how far the disease has progressed and the resistance of the case to the disease.

Observations during treatment

(a) It is probably impossible to produce pyrogen free water in this hospital. The still available for making distilled water is all metal. Pyrexia, often very high was recorded in every case under treatment. The temperature would rise rapidly about 1 hour after treatment had commenced and fall rapidly to normal at the completion of treatment. This pyrexia and hyperpyrexia may enhance the action of the tryparsamide of pyrotherapy in the treatment of syphilis. It is however exhausting and the deaths that occurred were probably due to this additional strain on a myocardium already damaged by the disease and other factors.

(b) Pain along the course of the vein used was common but did not constitute more than a temporary inconvenience to the patient.

(c) In three cases damage to the optic nerve resulted. Preliminary examination with an ophthalmoscope was not made as a routine before treatment was commenced. It is recommended that this should be done. In all three cases acuity of vision dropped to perception of light only. The optic disc became paler than normal. Ametox, 0.45 gramme in 10 c.c. was given daily for 5 days intravenously. In two of these cases vision improved considerably and was sufficient to allow the cases to pursue their vocations. One case has shown no improvement after 3 months. In one case visual impairment was noticed after 4 days treatment (8 grammes of tryparsamide), in another after 6 days (18 grammes), in the other after 6 days (12 grammes).

(d) Five cases showed a trace of bile in the urine at the completion of treatment. This was not associated with any other evidence of liver damage and cleared rapidly when treatment ceased. It is of interest to note that this complication occurred only when other cases of liver disease were present in the wards.

(e) The treatment caused much less disturbance and weakness in children in adults, although hyperpyrexia even up to 105° F was more common the children.

RESULTS OF TREATMENT

In all cases no trypanosomes were found in the blood or spinal fluid on completion of treatment with the exception of Case 16 In the twenty-seven previously G.P + the glandular enlargement had completely subsided. There was marked clinical improvement in all cases. The thirty-five cases who survived treatment were all symptom free and those previously somnolent had lost that characteristic and had regained an average mental alertness. It no exaggeration to say that all the cases were greatly pleased with their improvement in so short a space of time.

results

It is impossible to estimate the curative effect or otherwise of the treatwithout further examination of the cases over a period of 18 months. The results of the twenty four cases given below appear however to be These cases have been examined periodically over periods from 4 to 7 months since their original treatment. Eleven cases could not be traced for further examination.

CASES.

Case 2.—Male aged 7 years Weight 22 kg Symptom free. G.P = trypano-+ Blood negative. C.S.F negative. August, 1943 6 grammes tryparsamide 6 days. No toxic effects. April 1944 weight 22½ kg Symptom free. Looks and feels well No glands. C.S.F and blood negative.

Case 3—Male, aged 42 years Complains of somnolence. General condition poor Moderately ataxic. Dull mentally Weight 49 kg No glands. Blood negative. C.S.F., cells per c.mm. Trypanosomes present. August, 1943 16 grammes tryparsamide 6 days No toxic effects. 29 12.43 weight 82½ kg Symptom free. No ataxia. Mentally Now working as labourer No glands. Blood negative. C.S.F., 10 cells per c.mm trypanosomes. Albumin 0·028 per cent.

Case 4—Male, 38 years. Weight 47 kg Very ataxic. Markedly emotional. Conaphasic. Partially dysphasic. General condition poor Blood negative. G.P present. C.S.F., 140 cells per c.mm. Trypanosomes present. August, 14 grammes tryparsamide in 7 days. No toxic effects. November 1943 weight kg Blood negative. C.S.F., 56 cells per c.mm. No trypanosomes. Albumin 0·056 cent. Has lost the emotionalism and looks and feels better Not ataxic. No dysphagia. slow and slurred. Given further 10 grammes tryparsamide in 5 days with no effects. March, 1944, weight 54½ kg Feels and looks well. Still has slow slurred Blood negative. C.S.F., 23 cells per c.mm. No trypanosomes. Albumin 0·056 cent.

Case 6—Male, aged 7 years Weight 20 kg General condition good. Complains joint pains. Not somnolent. G.P positive. Blood negative. C.S.F., 6 cells. No August, 1943 6 grammes tryparsamide in 6 days. No toxic effects. March, symptom free. Feels and looks well. Weight 21 kg. No glands. Blood negative. C.S.F., 5 cells per c.mm. No trypanosomes. Albumin 0·01 per cent.

Case 7—Male, aged 9 years. Weight 24 kg Joint pains. Not somnolent. General condition good. G.P trypanosomes present. Blood negative. C.S.F., 2 cells per c.mm.

No trypanosomes. August, 1943 6 grammes tryparsamide in 6 days. March 20, symptom free. Looks and feels well. Weight 27½ kg. Blood negative. C.S.F 5 cells per c.mm. No trypanosomes. Albumin, 0·01 per cent.

Case 8.—Male, aged 44. Weight 70 kg. Joint pains. Very ataxic. General physics good. Not somnolent. G.P. positive. Blood negative C.S.F., 14 cells per c.mm. So trypanosomes. September 1943 18 grammes tryparsamide in 8 days. On the 6th day of treatment his vision became poor. He was given 0·045 gramme sodium hyposulphite daily intravenously for 5 days. There was considerable improvement in visual acuity There was also a slight trace of bile in the urine for 3 days from the 6th day of treatment. December 1943 slightly ataxic. Feels and looks well. Weight 71½ kg. Blood negative C.S.F. 10 cells per c.mm. No trypanosomes. Albumin 0·038 per cent. Refuses further treatment as he is afraid of losing his eyesight

Case 13.—Male aged 25 years. Insane. Very noisy and difficult to control. Talks incessantly. Tends to be violent. Weight 53 kg. General condition fair. G.P. positive. Blood negative. C.S.F 204 cells. Trypanosomes present. September 1943, 20 grammes tryparsamide in 7 days. December 1943 much improved. Now quiet and easy to control. Is euphoric and talkative. Blood negative. C.S.F., 10 cells per c.mm. No trypanosomes. Albumin 0·02 per cent.

Case 14.—Male, aged 62 years. Weight 54 kg. Complains of joint pains. General condition good. G.P. trypanosomes present. Blood negative. C.S.F., 10 cells per c.mm. No trypanosomes. September 1943 12 grammes tryparsamide in 6 days. No toxic effects. April 1944 symptom free. Feels and looks well. Weight 56 kg. Blood negative. C.S.F 6 cells per c.mm. No trypanosomes. Albumin 0·016 per cent.

Case 15.—Female aged 10 years. Weight 29 kg. Slight somnolence and generalised pains. General condition good. G.P. and blood negative. C.S.F., 24 cells per c.mm. No trypanosomes. September 1943 7½ grammes tryparsamide in 6 days. No toxic effects. December 1943 symptom free. C.S.F. 12 cells per c.mm. No trypanosomes. Albumin 0·03 per cent. Given 8 grammes tryparsamide in 7 days. April 1944 Symptom free. Feels and looks well. Weight 31 kg. Blood negative C.S.F., 10 cells per c.mm. No trypanosomes. Albumin 0·02 per cent.

Case 16.—Female, aged 9 years. Weight 26 kg. General condition good. Very emotional, stupid and somnolent. G.P. positive. Blood negative. C.S.F. 410 cells per c.mm. Trypanosomes present. September 1943 7½ grammes tryparsamide in 6 days. No toxic effects. December 1943 Weight 30 kg. Shows general improvement. C.S.F 210 cells per c.mm. Trypanosomes present. Albumin 0·03 per cent. 6 grammes tryparsamide in 4 days given. No toxic effects. January 1944 emotionally stable. somnolent. Feels and looks well. April, 1944 weight 30 kg. Appears very well. Blood negative. Spinal puncture not successful.

Case 17.—Male, aged 18 years. Generalised pains and somnolence. Weight 40 kg. General condition good. No glands. Blood negative. C.S.F., 14 cells per c.mm. No trypanosomes. September 1943, 9 grammes tryparsamide in 6 days. No toxic effects. March, 1944 Weight 83 kg. Symptom free. Looks and feels well. Blood negative C.S.F., 2 cells per c.mm. No trypanosomes. Albumin 0·01 per cent.

Case 18.—Female, aged 25 years. Symptom free. G.P. and blood positive. C.S.F 2 cells per c.mm. No trypanosomes. Weight 82 kg September, 1943, 12 grammes tryparsamide in 8 days. No toxic effects. April, 1944, feels and looks well. Symptom free. Blood negative. C.S.F 2 cells per c.mm. No trypanosomes. Albumin 0·016 per cent.

Case 19.—Male, aged 30 years. Generalised pains, headache and somnolence. General condition good. Weight 45 kg. G.P. negative. Blood positive. C.S.F 270 cells per c.mm. No trypanosomes. October 1943 12 grammes tryparsamide in 6 days. No toxic effects. December 1943, feels and looks well. Weight 45½ kg. C.S.F 15 cells per c.mm. No trypanosomes. Albumin 0·033 per cent. 8 grammes tryparsamide in 4 days April, 1944 working now as ferry boy. Feels and looks well. No symptoms. Weight 45 kg. Blood negative. C.S.F., 6 cells per c.mm. No trypanosomes.

Case 21.—Male, aged 50 years. General weakness. Very thin. Very emaciated

Moderately somnolent. Unable to stand without support. G.P. and blood negative. C.S.F., 20 cells per c.mm. No trypanosomes. Weight, 48 kg   October 1943 12 grammes tryparsamide over 12 days, each dose being 2 grammes (six injections only) but treatment interrupted to rest the case as he was very weak) 26 10 43 C.S.F., 70 cells per c.mm. No trypanosomes. Albumin 0·06 per cent 6 grammes tryparsamide in 3 days. 18 11 43 C.S.F., 10 cells per c.mm. No trypanosomes. Albumin 0·03S per cent. 2.12.43 can now walk. Is sensible Not sleepy No symptoms 7 1 44 returned to farm work Weight 46½ kg February 1944 C.S.F 30 cells per c.mm. No trypanosomes. Albumin 0·03 per cent 12 grammes tryparsamide in 2-gramme doses over 9 days No toxic effects. April, 1944 looks and feels well Symptom free. Blood negative. C.S F., 20 cells per c.mm. No trypanosomes. Albumin 0·03 per cent. Weight, 54½ kg

Case 22—Male aged 16 years. Thoracic pain. General condition good. Weight 44 kg G.P. negative. Blood negative. C.S.F 2 cells per c.mm. No trypanosomes. October 1943 12 grammes tryparsamide in 6 days No toxic effects. 31.3 44 feels and looks well. No symptoms Weight 51½ kg C.S.F., 5 cells per c.mm. No trypanosomes Albumin, 0·018 per cent.

Case 23—Female, aged 35 years. Somnolence. General condition good Weight 49 kg G.P and blood negative. C.S.F 40 cells per c.mm. No trypanosomes. October 1943 12 grammes tryparsamide in 6 days 18 11 43 feels well. C.S.F., 20 cells per c.mm. No trypanosomes. Albumin 0·02 per cent Tryparsamide 6 grammes in 3 days. 22.3 44 Symptom free Feels and looks well. Now pregnant. Blood negative. C.S F 6 cells per c.mm. No trypanosomes. Albumin 0·02 per cent. Weight 53 kg

Case 24—Male, aged 6 years Mother states that the boy is sleepy General condition fair Weight 21 kg G.P and blood negative C.S F. 24 cells per c.mm. No trypanosomes. October 1943 6 grammes tryparsamide in 6 days March, 1944 no symptoms. Feels and looks well. Blood negative. C.S F 6 cells per c.mm. No trypanosomes. Albumin 0·01 per cent. Weight 22½ kg

Case 25—Female aged 50 years. No symptoms. G.P positive. Blood negative C.S.F., 10 cells per c.mm. No trypanosomes Weight 46 kg October 1943 12 grammes tryparsamide in 6 days No toxic effects. March, 1944 feels and looks well. Weight 46 kg Blood negative. C.S F 8 cells No trypanosomes. Albumin 0·02 per cent.

Case 26—Male aged 28 years. Complains of somnolence Very drowsy and dull Staggers as he walks General condition poor Weight 61 kg G.P positive. Blood negative C.S F 450 cells. Trypanosomes present. Albumin 0·04 per cent. October 1943 14 grammes tryparsamide in 6 days No toxic effects. November 1943 C.S.F., 30 cells per c.mm. No trypanosomes. Albumin 0·037 per cent. No longer sleepy Feels and looks improved. 6 grammes tryparsamide in 4 days. 23.3 44 symptom free. Mentally alert. Weight 61 kg C.S F., 20 cells per c.mm. No trypanosomes. Albumin 0·022 per cent. Blood negative.

Case 28—Male, aged 40 years Headache and generalized pains. General condition fair G.P positive. Blood negative. C.S.F., 10 cells per c.mm. No trypanosomes. Albumin 0·01 per cent. Weight 56 kg November 1943, 8 grammes tryparsamide in 4 days. Rested for 2 days owing to appearance of bile in urine. Then further 4 grammes in 2 days. March, 1944 symptom free. Looks well. Weight 59 kg Blood negative C.S F 10 cells per c.mm. No trypanosomes. Albumin 0·015 per cent.

Case 32—Female, aged 39 years. Headache and generalized pains. General condition good. Weight 41 kg G.P positive Blood negative. C.S.F., 2 cells per c.mm. No trypanosomes. Albumin 0·01 per cent. November 1943 12 grammes tryparsamide in 6 days. No toxic effects March, 1944 feels and looks well. Weight 42 kg Blood negative. C.S.F., 5 cells per c.mm. No trypanosomes. Albumin 0·01 per cent.

Case 35—Male aged 18 years. Headache and somnolence. Very dull and sleepy Slightly ataxic. Weight 45 kg General condition poor G.P and blood positive. C.S.F. 1 420 cells per c.mm. No trypanosomes. Albumin 0·056 per cent. November 1943 between 6th and 20th November given 16 grammes tryparsamide in doses of 2 grammes daily on eight of these days. No toxic effects March, 1944 feels and looks well. No ataxia. Mentally alert. Weight 50 kg Blood negative C.S.F., 8 cells per c.mm. No trypanosomes Albumin 0·022 per cent. No trypanosomes

Case 38—Female aged 8 years. Somnolence. Very emotional. Weight 22½ kg. General condition fair. G.P. and blood positive. C.S.F., 30 cells. No trypanosomes. Albumin 0·03 per cent. December 1943 7 grammes tryparsamide in 7 days. No toxic effects. March, 1944 symptom free. Looks well. No longer excitable. Weight 25½ kg. Blood negative. C.S.F., 6 cells. No trypanosomes. Albumin 0·019 per cent.

Case 39—Male, aged 35 years. Complains of somnolence. Very stupid. General condition poor. Weight 46½ kg. No glands. Blood negative. C.S.F., 110 cells per c.mm. No trypanosomes. Albumin 0·048 per cent. December 1943 6 grammes tryparsamide in 3 days—2 days rest, followed by further 6 grammes in 3 days. At completion of treatment he complained of dimness of vision. Visual acuity light perception only, and thus has not improved after 3 months. He was treated with ametroin. April, 1944 symptom free. Weight 50 kg. No longer sleepy. Blood negative. C.S.F., 10 cells per c.mm. No trypanosomes. Albumin 0·03 per cent.

Conclusions.

1. It has been established that it is possible to give tryparsamide by massive dose intravenous drip method.

2. The treatment requires hospitalization but reduces the time of treatment from 12 weeks or more to about 12 days or less.

3. The method produces rapid sterilization of the blood and glands of trypanosomes and it is unlikely that treated cases will become arsenic resistant.

4. Children and young adults tolerate the treatment well. The aged are unsuitable for such treatment.

5. It would be of interest to observe the effects of this treatment, using pyrogen-free water and substituting reduced tryparsamide for tryparsamide.

Summary

Forty two cases of trypanosomiasis in varying stages of the disease, treated with massive dose tryparsamide by intravenous drip method, are described with brief case histories of twenty four cases examined 4 to 7 months after treatment.

CORRESPONDENCE

SPRAY KILLING OF MOSQUITOES

Sir,

Anti-malarial spraying* is a most valuable measure, but cannot yet everywhere replace screened houses and anti larval operations, although *everywhere* it is a splendid adjunct. The new insecticide, DDT may transform the situation The 1 lb Westinghouse aerosol 'bomb' using freon (and perhaps DDT) is a most valuable weapon. Petrol driven motor paint spray guns have been used with kerosene-pyrethrin mixtures very successfully where houses—and not tents—were the dwellings treated. We got over the difficulty of locked rooms by having a long thin nozzle made that could be poked through a keyhole, or through a small hole specially drilled in the door and normally covered by a small pivoted flap. Freon is rather hard to get and is also used for air conditioning plant but, *when there is no fire risk* butane is an equally good dispenser.

One of the great advantages of any kind of spraying technique is that adult anopheles can be counted and perhaps dissected, thus affording a valuable control for anti malaria methods from year to year. Counting the stunned or apparently lifeless bodies of mosquitoes is made easier if the manoeuvre of Mr A. Hussey (late Health Inspector A.I O C. is used. He spread a large white sheet over the open doorway of the room—after blocking up all chimneys, ventilators windows and other potential escape routes—and then started spraying. The intoxicated insects flew into the shining white sheet and fell on to a newspaper spread on the floor at the bottom of the sheet. Subsequent counting identification, dissection, state of ovaries, stomach blood precipitation tests etc. were simplified. Floor sweeping was unnecessary.

With regard to Professor Blacklock's remarks about adult mosquitoes sheltering in vegetation near dwellings, we found that French marigolds (*Tagetes patula*)—the variety known as 'Legion of Honour" a single golden flower with a dark brown velvety centre and dark green fern-like leaves—

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*Entry L. G (1944) " Spray Killing of Mosquitoes in Houses A contribution to Malaria Control on the Gold Coast." *Trans R. Soc trop Med. Hyg.*, 38 (3,) 167

provided eagerly sought refuges for anopheline and culicine adults in hot weather when adults were difficult or impossible to find in their usual shelter. Gardeners like sowing these flowers in tight clumps and the ground in the middle of the clump is always damp even in very hot dry Mesopotamian summers

The native population never objected to spraying of their dwellings, they appreciated the killing of flies more than mosquitoes both, of course, were involved in the holocaust.

An ingenious trap for mosquitoes and flies was developed by Mr HUGH and Mr C BROOKING (both Health Inspectors) They sprayed suitable vegetation—plants and bushes, never grass—with a dilute solution of sodium arsenite and molasses which proved a very potent and effective contact poison even when dry

I am, etc.,

Abadan Iran. FRANK MEEK

TREATMENT OF BILHARZIASIS WITH STILBAMIDINE.

SIR,

The late Professor WARRINGTON YORKE suggested that a trial should be made to study the effect of stilbamidine in the treatment of bilharziasis. A series of nine cases has now been treated and, though the results do not justify further trial they may be of interest to other workers.

Cases heavily infected with *Schistosoma haematobium* were selected as the effect of treatment can be more easily observed with this type than with *S mansoni*

The effect of treatment was judged solely by the effect on the eggs, a presumption of cure only being made when dead eggs, from which miracidia could not be hatched were passed. It was for this reason that heavily infected cases were selected for the absence of eggs from the urine cannot be taken as a criterion of cure since periodic variations in the number of eggs passed occur irrespective of treatment, and in lighter infections periods may occur in which no eggs can be discovered in the urine.

The drug used was 4 4 diamidinostilbene-di-isethionate (stilbamidine) and the dosage used was similar to that used by KIRK and SATTI* in their treatment of kala-azar due allowance being made for the difference in the compound used. The average adult course of fifteen injections varied from 2·0 to 2·9 grammes,

In five of the cases, there was no apparent change in the condition after one course of fifteen injections.

* KIRK, R. & SATTI (1940). *Ann. trop. Med. & Parasit.*, 34 85.

In two cases the urine increased in quantity became much clearer and the number of eggs passed was greatly diminished. Hatching of miracidia was still observed, and it is therefore uncertain whether the observed effect was due to the drug or not.

The remaining two cases were presumed cured, the effect of the drug becoming apparent early in treatment.

In one, the urine had cleared considerably and the number of eggs diminished after only five injections Miracidia could only be hatched from about one in ten eggs, and when hatched their movements were sluggish. Of the other eggs, some were black and shrivelled others appeared normal but no movement could be detected in the contained miracidia.

Before the end of the course no miracidia could be hatched from the eggs passed. The patient was observed for another month, at the end of which time the urine was clear and no eggs could be found.

The other was the first case in which this treatment was tried and the result was somewhat dramatic.

The patient came to hospital complaining of swelling of both testes, and irregular attacks of fever for 10 months. The right testis was enlarged to about twice its normal size, the epididymus being more affected than the body of the gland. It was hard, heavy and painful The cord was thickened and irregular The left testis was affected but to a lesser degree The prostate was not enlarged. There was a large tender mass of iliac glands on each side. The spleen and liver were both enlarged. The urine appeared to be almost pure mucus, streaked with blood, and contained an enormous number of S haematobium eggs twenty to thirty being found in a drop of uncentrifuged urine under a cover slip

Within 3 days of the commencement of treatment a change became noticeable in his urine It had cleared considerably and the number of eggs was diminishing By the end of the course of fifteen injections his general and local condition was greatly improved. His urine contained only a little mucoid deposit and the eggs were scanty From some, miracidia could be hatched, while others were negative, and there was quite a large proportion of black and shrivelled eggs.

A second course of ten injections was given after an interval of 10 days, after the third of which no miracidia could be hatched from the few eggs passed in the urine.

When the patient was discharged from hospital, 7 weeks from the date of admission, his urine contained a slight deposit, the left testis and cord appeared normal, while there still remained some nodular enlargement of the right epididymus. The iliac glands had diminished considerably and were no longer tender The spleen and liver remained unchanged.

The patient was kept under observation for a further 2 months during which time there was no relapse and no eggs were found in his urine.

Summing up it would appear that stilbamidine is of therapeutic value in some cases of schistosomiasis but, in the writer's opinion, this effect is too uncertain to justify the use of the drug as a routine treatment.

I am, etc.,

R. W. STEPHENSON,
Senior Medical Inspector
Sudan Medical Service.

Abu Usher Sudan.

A CASE OF BLACKWATER FEVER IN AN AFRICAN GIRL[*]

SIR,

In this paper Dr SHIRCORE says "Nor can I recollect the record of any bacteriological examination of the urine and blood in the literature of blackwater fever."

Has Dr SHIRCORE forgotten the following among other records, or is it that he has overlooked them?

Blood.—(a) "In two cases cultures were made from the blood on agar; both they remained sterile. In one case cultures were made from the spleen and heart's blood in both pure cultures of *Staphylococcus aureus* resulted. STEPHENS, J W W and CHRISTOPHERS S R. (1900) *Reports to the Malaria Committee of the Royal Society.* 23

(b) "In two cases I have found streptococcus-like organisms in the blood. CRICHLOW N. (1929). The prevalent diseases of the British Solomon Islands. *Trans R. Soc trop Med. Hyg.*, 23 179-184

(c) "BOINET has in Indo-China described a streptococcus which he found in the blood of a patient for a long time suffering from malaria and attacked with blackwater fever." CARDAMATIS, J P (1902b) De la fievre bilieuse hémoglobinunique observee en Grèce. *Prog Med.*, Nos. 37-40

Urine.—(a) Streptococci present in the majority of urines." ROSS G R. (1932) Researches on blackwater fever in Southern Rhodesia. *Liv Sch Hyg trop Med.*, Mem. 6, 196.

(b) Cases, twenty-one. Organisms in twenty staphylococci in nineteen streptococci in one, various in five. Control cases, forty four organisms in thirty four staphylococci in thirty streptococci in seven, various in six. GORDON R. M. and DAVEY T H. (1935). The association of bacteria with blackwater fever in West Africa. *Ann trop Med. Parasit.*, 29, 439-456

I am, etc.,

J. W. W. STEPHENS

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* SHIRCORE, J O (1944) *Trans. R. Soc trop. Med. Hyg.*, 38, (2), 161.

Sir,

We were interested to read in the November number of the TRANSACTIONS the report by Dr SHIRCORE of a case of blackwater fever associated with streptococci in the urine, which improved rapidly after the administration of sulphathiazole. Judging by the article, and by the cable subsequently sent by Dr SHIRCORE to the editor Dr SHIRCORE would appear to suggest that the streptococcus was at any rate in part, the cause of the haemoglobinuria He writes 'It is deplorable that of all the above cases—except the present one—none of the urinary deposits was stained and examined for bacteria and of course, as a consequence, it is now impossible to correlate the present findings with any previous example nor can I recollect the record of any bacteriological examination of the urine and blood in the literature of blackwater fever The bearing of a haemolytic streptococcal infection of the renal pelvis, with the probability of a concurrent septicaemia on the aetiology and mechanism of blackwater, certainly demands close investigation, by cultural methods including blood culture, for it is not beyond the realms of possibility that an organism of this nature might be a contributory factor if not the cause, of blackwater fever, in the malarial subject. Dr SHIRCORE would appear to have missed an article by us (GORDON and DAVEY 1935) which records the association of bacteriuria with blackwater fever and describes eighty two strains of organisms isolated from a series of blackwater and control cases examined in West Africa. As it seems to bear directly on this important subject, it may be worth while quoting in full the summary and conclusions which we published.

SUMMARY AND CONCLUSIONS

In seven cases of blackwater fever examined during the active stage of the disease all seven were found to be excreting organisms in their urine Amongst so small a series of cases such an association may of course, be merely a coincidence we cannot however dismiss it as such, until a case of active blackwater fever is encountered in whose urine no organisms are to be found. We have not observed such a case in Sierra Leone, and the one case examined for us in England was also associated with a bacteriuria.*

In thirteen recovered cases, examined at various periods from 1 month to 10 years after the attack, twelve were found to have bacteria in their urine, while in one, examined 2 years after the attack, no bacteria were found on culture.

Amongst forty-four European and African controls, examined by the same technique thirty-four were found to be similarly excreting organisms. The incidence of bacteriuria, therefore, amongst the active and recovered blackwater cases was higher than amongst the controls 95 per cent. as compared with 77 per cent.

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*Subsequently another case of active blackwater was examined by one of us in England and found to be passing a heavy concentration of staphylococci.

" In the small series of active and recovered blackwater cases in which quantitative estimations were made, the concentration of bacteria was generally found to be higher than amongst the control cases

" A comparison of organisms of the same genera, i.e., staphylococci or streptococci, isolated from active and recovered blackwater cases and from control cases showed no essential differences in morphological, cultural or biochemical characters.

We have been unable to produce any evidence that bacteria isolated from the urines of active and recovered blackwater cases or from control cases show any marked pathogenicity.

Although it has thus been shown that blackwater fever is constantly associated with bacteriuria in the series of cases examined by us and that the concentration of bacteria is usually high, yet, since we have been unable to demonstrate any marked pathogenicity in the organisms isolated, and since controls show that bacteriuria is widespread amongst Europeans and Africans in Sierra Leone it follows that its mere presence in cases of blackwater fever cannot be regarded as evidence that it bears any causal relationship to the disease. Further the results obtained prove that a combination of chronic malaria undergoing treatment with quinine and associated with bacteriuria does not necessarily result in blackwater fever for several of our European control cases presented this combination, although in these cases the concentration of bacteria was low   In addition, some of the controls who at the time of examination were similarly excreting a small number of bacteria in their urine but were not suffering from malaria, subsequently contracted malaria without developing haemoglobinuria.

The remarkable high bacterial concentration which we have recorded in some of our active and recovered blackwater cases is the only feature which we have not observed in the series of control cases. Whether the high bacterial concentration in the urine in these cases was a mere coincidence, or whether it played any part in the causation of the disease, cannot be determined without examining a large number of cases."

<div style="text-align:right">

We are etc.,

R. M. GORDON

T. H. DAVEY
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Liverpool School of Tropical Medicine

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TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL. XXXVIII. No 5   MAY 1945

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W

on

Thursday, 18th January, 1945, at 8 p m

THE PRESIDENT

SIR HAROLD SCOTT K C M.G M.D F.R.C.P F.R.S.E.

in the Chair

PAPER

CHEMOTHERAPEUTIC SUPPRESSION AND PROPHYLAXIS
IN MALARIA.

AN EXPERIMENTAL INVESTIGATION UNDERTAKEN BY
MEDICAL RESEARCH TEAMS* IN AUSTRALIA.

BY

Brigadier N HAMILTON FAIRLEY C.B.E., F.R.S.,
Director of Medicine Australian Military Forces

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* Medical and Science Personnel.—(1) L H Q *Medical Research Unit*—O C
Lieut.-Colonel R R ANDREW later Lieut. Colonel C R BICKERTON BLACKBURN *Entomology* Major M. J MACKERRAS Major F H S ROBERTS and Major S L W ALLMAN
(temporary) *Pathology* Major T C BACKHOUSE, Major T S GREGORY Lieut. A C
POPE, Lieut. S R. DUNN (2) *Inland Australian General Hospital*—O C Lieut.-Colonel
L J WOOD later Lieut -Colonel R R ANDREW *Clinical* Major J PEDLER Captain L.
FORSYTH *Pathology* Major J TONGE Major A A FERRIS Captain M. J FREEMAN
Blood Transfusion Major R. OFFICER Sister E M. ROBERTS *Convalescent Depot* Major
R. WHEELER, Captain B BRENTON

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This report is of a preliminary nature and no references to the literature are being made at the present juncture. It is proposed to publish a final report with an adequate bibliography on these and newer anti-malarial drugs at a later date when security considerations permit.

INTRODUCTION

MALARIA IN THE SOUTH WEST PACIFIC (1942-1943)

The first severe malaria casualties in the South West Pacific comprised a group of Australian infantry who had retreated from Rabaul after its fall to the Japanese in January 1942. With a medical officer who was sagacious enough to collect what quinine he could some 252 men crossed the jungles of New Britain, finally escaped to New Guinea and later crossed to the Australian mainland   For the 1st month in New Britain enough quinine was available to treat sick malaria casualties   Then quinine supplies became exhausted and in the next 4 or 5 weeks fifty men died from malignant tertian malaria.   On reaching the mainland the remainder were found to be suffering from mixed malignant tertian (M.T) and benign tertian (B T) infections. The malignant tertian malaria was readily cured and the benign tertian attacks responded satisfactorily to standard treatment, the temperature subsiding and parasites disappearing but relapses occurred frequently at short intervals and anti malarial drugs failed to cure the infection

Unfortunately subsequent experience confirmed these early findings and it soon became evident that the strain or strains of *Plasmodium vivax* acquired in New Britain and New Guinea differed from the strains of *P vivax* which had affected Australian troops in Egypt and Syria during 1940 to 1942 in two respects (1) though the immediate response to anti malaria drugs was satisfactory the relapse rate was very high   (2) relapses occurred with great regularity within 4 to 8 weeks of the primary attack or the cessation of atebrin therapy   The long period of 6 to 9 months which had been frequently noted to intervene between the first attack of fever in the Middle East in *P vivax* infections and the first relapse, was conspicuous by its absence   This led in Australia to the adoption of a maintenance dose of atebrin for 6 weeks after

standard treatment in order to postpone relapses. This relieved the hospital bed state and enabled infected troops to be rehabilitated and retrained prior to returning to hyperendemic areas.

Throughout the various campaigns in the South-West Pacific, malarial casualties in combat zones have far exceeded battle casualties, a ratio of from 5 to 30 to 1 being commonly encountered. This held at Guadalcanal as well as in the various New Guinea campaigns, including Milne Bay, Buna-Gona and fighting in the Markham and Ramu Valleys and the Huon Peninsula.

From a man-power viewpoint the outstanding problems of military importance in the South-Western Pacific Area have been (1) the control of excessive malaria casualties during a war of movement in highly malarious country (2) the control of relapses of benign tertian malaria in infected troops from malarious areas who had returned to Australia and ceased taking suppressive atebrin.

Owing to the urgency of the malaria problem in the South-Western Pacific Area, the C in C acting on the advice of the D G M S, decided to establish in tropical Queensland two research groups under my direction (1) a L.H.Q Medical Research Unit attached to an Australian General Hospital in the coastal area (2) a Research Group comprising specially selected medical and laboratory personnel at another Australian General Hospital working in conjunction with a Convalescent Depot further inland. The unit objective was to investigate the mode of action and the precise value of anti-malaria drugs including certain new sulphonamides, atebrin and quinine as suppressives and true causal prophylactics in volunteers infected with Papuan strains of *P vivax* and *P falciparum*. Since then a number of new anti malarial drugs have been tested but for security reasons the results obtained cannot yet be reviewed.

The general plan was to expose volunteers, taking anti-malarial drugs in a specified daily dosage, to bites of malaria-infected mosquitoes at L.H.Q Medical Research Unit and to study them there throughout the incubation period of malaria. Subsequently these volunteers were to be sent inland to the other group of research workers at another Australian General Hospital, where investigations would be continued to determine whether the drug in question had merely suppressed the infection or actually cured the patient.

I MEDICAL RESEARCH PERSONNEL AND PLAN OF INVESTIGATION.

(A) L H Q MEDICAL RESEARCH UNIT

The L H Q Medical Research Unit, established in June 1943, consisted of entomological, pathological and clinical sections and was accommodated in an Australian General Hospital near the coast where the annual rainfall averaged 88·4 inches. An entomological laboratory, a laboratory for parasitological, pathological and biochemical investigation and screened wards adequate to

accommodate 120 volunteer patients and malaria carriers were ultimately allocated for medical research purposes. The first officer commanding the unit was Lieut. Colonel R. R. ANDREW later Lieut. Colonel R. BICKERTON BLACKBURN took over

1 Entomological Section

Major F. H. S ROBERTS and the staff of two Australian Mobile Entomological Sections, assisted by Major M J MACKERRAS began the work of providing a supply of infected mosquitoes Subsequently Major S L ALLMAN, with the staff of another Australian Mobile Entomological Section, assisted in carrying on this work with Major MACKERRAS who for the past year has been in sole charge of this Section.

The function of the entomological team was (a) to collect larvae of anopheline mosquitoes which were known to be vectors of malaria in the South-Western Pacific Area (b) breed out and maintain the adult females (c) feed the females on selected gametocyte carriers of malignant or benign tertian malaria (d) transmit malaria by subjecting volunteers to a known number of bites from mosquitoes, the sporozoite rate of which had been determined by dissection and microscopic examination of the salivary glands.

During the early period of these experiments conditions were very dry and larvae had to be collected and transported by air many hundreds of miles from areas as widely separated as South Queensland and New Guinea. The species aimed at were *A punctulatus* var *typicus A punctulatus* var *moluccensis* and *A annulipes* which are established vectors of malaria in Australia or New Guinea. The species which survived best under laboratory conditions hit most effectively had a high average infection rate and proved to be the most efficient all-round transmitter was *A punctulatus* var *typicus* and this species has been mainly used for the experimental transmission of malaria in the later experiments A special collecting unit is now stationed in New Guinea for this purpose some 20 000 larvae being transported by air from there to L.H.Q Medical Research Unit every week unless adverse weather prevents this being done

A serious difficulty concerned gametocyte carriers the dearth of suitable carriers was undoubtedly related to the long periods of time during which troops were taking atebrin for suppressive purposes and as a maintenance dosage after routine hospital treatment. Three sources for the supply of carriers were used (1) hospitals in New Guinea, suitable patients being at first flown to the mainland (*P falciparum*) (2) hospitals in Northern Queensland (*P vivax* and *P falciparum*) (3) volunteers experimentally infected with malaria parasites transmitted by mosquito bite or blood inoculation (*P falciparum*)

The gametocytes were counted in thick films stained by Field s method and the number occurring on an average in ten fields was given as the gametocyte

rate With *P. vivax* a rate of 2 or more was regarded as satisfactory. With *P. falciparum* slightly higher rates were necessary from 5 to 110 proved satisfactory. Estimation of the number of gametocytes per c.mm. were also made by (1) SINTON's fowl cell method, (2) estimating the total leucocytes and counting the number of gametocytes against the leucocytes in blood film, (3) the loop method in thick smears introduced by Major BACKHOUSE. A general relation was found between the number of gametocytes and the infection rate the higher the gametocyte count the greater the likelihood of getting a large percentage of mosquitoes infected. There were, however exceptions to this rule and complete failure to infect batches of mosquitoes sometimes occurred for unknown reasons after feeding on carriers in whom the gametocyte density the gametocyte sex ratio maturity and exflagellation in the thick gametocyte appeared satisfactory. Infective rates in mosquitoes varied from nil to 100 per cent. A 50 per cent. infection was regarded as reasonable. Medium to heavy infection of the salivary glands with sporozoites was the rule. Generally the calculated number of infective bites was not less than three thus if 50 per cent. of the batch were infected, biting would be stopped after six mosquitoes had engorged.

2. *Pathological Laboratory Section*

The officer commanding the pathological laboratory in the first instance was Major T. C. BACKHOUSE, later he was succeeded by Major T. S. GREGORY. The object of the various investigations was to determine the effects of the daily consumption of anti-malarial drugs in specified doses on —

(1) Malarial parasites introduced as sporozoites into healthy volunteers by controlled biting of infected mosquitoes.

(2) The health of volunteers as judged by the result of certain clinical laboratory tests.

The routine pathological investigations included —

(*a*) Daily examination of thick blood films stained by Field's method. From 1 to 2 c.mm. of blood were searched for parasites—a very laborious and time-consuming procedure.

(*b*) Haemoglobin estimations and erythrocyte counts.

(*c*) Total and differential leucocyte counts.

(*d*) Urine examinations.

(*e*) Estimations of drug concentration attained in the plasma.

Drug Estimations —The procedure adopted for the analysis of plasma for atebrin was that developed by BRODIE and UDENFRIEND a Coleman photofluorometer (Model No. 12) being used for this purpose. Some of the earlier atebrin estimations included in the present report, were made by Lieutenant BANG and Lieutenant TRAGER, U.S.A. M.C. Later all quinine and atebrin estimations in plasma were made in the laboratory of the L.H.Q. Medical Research Unit.

Estimations of the sulphonamide levels in the blood were made mainly by Fantl's modification of Werner's method. Marshall's method possesses certain advantages and has also been used in a proportion of later estimations.

3 Clinical Section

Volunteers were restricted to men who had never lived in malarious areas. Volunteers had to (1) be organically sound, physically fit and mentally stable (2) have no crime record (3) be free from venereal disease (4) not have suffered from asthma (5) not have been jaundiced during the preceding year.

The function of the Clinical Section was (1) the care of the experimentally infected volunteer and the gametocyte carrier (2) supervision of drug administration (3) the clinical study of the patient and the daily recording of clinical data including temperature readings presence of herpes splenomegaly hepatomegaly anaemia, jaundice etc. (4) if malaria should break through, the treatment of primary malaria attacks by different modes of therapy and a careful follow up to determine the subsequent relapse rate (5) the artificial production of carriers. When gametocyte carriers were scarce treatment was sometimes withheld for some time and modified in a manner calculated to exert minimum interference with gametocyte production following the primary trophozoite wave.

The various groups being tested always contained controls who received no anti malarial drugs, the remainder received daily doses of the drugs under strict medical supervision. In most groups the drug was administered daily for 23 days after the last infective bite, this period being selected as adequate to cover the normal incubation period of primary malaria. If the bitten volunteers failed to develop clinical malaria during this period they were generally sent inland for further observation and investigation as detailed below.

(B) 2ND RESEARCH GROUP AT AN INLAND AUSTRALIAN GENERAL HOSPITAL AND CONVALESCENT DEPOT

For purposes of this part of the investigation a special research group comprising clinicians, pathologists and biochemists was organized from staff personnel under Lieut. Colonel I J Wood O C Medical Division. A special research ward of sixty beds was allocated and arrangements made to ensure continuity of observation and records by maintaining medical and nursing personnel on as permanent a basis as possible. Adjacent to the hospital was a Convalescent Depot where excellent facilities existed for observation of malaria-infected volunteers following discontinuances of suppressive treatment. Later Lieut. Colonel R. R. Andrew became O C Medical Division. The main function of this research group was to investigate —

(1) The mode of action of anti malaria drugs in volunteers experimentally infected with trophozoite-induced malaria (blood inoculation)

(2) Those sporozoite-infected volunteers in whom malaria had not broken through while taking anti malarial drugs at L.H.Q. Medical Research Unit

The scheme of clinical observation and laboratory investigation was very similar to that already outlined. Special investigations were carried out to detect (a) latent malaria (b) susceptibility and premunity

In routine tests on these volunteers, some 23 days after exposure to infective bites or after the inoculation of blood containing malaria parasites, suppressant treatment had been discontinued. They were then observed for a further 5 weeks either at the Convalescent Depot or in the research wards of the Island Hospital. Swimming and cricket were included in routine exercises taken during this period, while, in addition, those at the Convalescent Depot had graded route marches.

(a) *Detection of Latent Malaria* —Where a volunteer failed to show a complete break through after this 5-week period, 200 c.c. of his blood were injected intravenously into a compatible recipient (volunteer). At first citrated blood was used. Later blood was directly transfused from donor to recipient by employing the direct blood transfusion apparatus devised by JULIAN SMITH for this purpose

In *P. falciparum* infection, if the recipient remained afebrile and malaria parasites failed to appear during the next 23 days, this was considered as definite evidence that latent malaria was not present in the donor. Such a result could be caused by (1) the non appearance of erythrocyte parasites in the donor due to the action of the drug on sporozoites or early tissue forms (2) destruction of erythrocyte parasites by the drug after they had appeared (3) host immunity preventing establishment of the infection

If erythrocyte parasites had been previously found in thick blood smears or demonstrated by subinoculation in the early stages of the infection, then it was evident that cure had been attained by destruction of asexual parasites Similarly host immunity could be excluded by susceptibility tests as described below

In *P. vivax* infections however a negative subinoculation test was not found to constitute reliable evidence of cure, since in the latent phase, subinoculation may fail to induce malaria in the recipient, yet the donor may subsequently develop overt B.T malaria. As indicated later this is to be regarded as evidence of persistence of tissue forms of *P. vivax* in contradistinction to *P. falciparum* where the tissue cycle is probably only of short duration

(b) *Susceptibility Tests (Natural Immunity).*—To exclude the presence of natural immunity or insusceptibility in the original test volunteer who had not developed malaria, he was given an intramuscular inoculation of some 10 to 20 c.c of blood obtained from a malaria donor infected with the same species of parasite as that originally injected by the mosquito or contained in the original experimental blood inoculum. The average parasite density in the blood was determined and the intramuscular dose containing from

100 to 800 million parasites adjusted accordingly No evidence of insusceptibility or natural immunity was ever demonstrated in volunteers either infected by mosquitoes experimentally or by the inoculation of blood containing malaria parasites. These tests also showed that the original test volunteer possessed no effective premunity to the particular strain of *P vivax* or *P falciparum* contained in the inoculated blood.

By subinoculation and susceptibility testing in *P falciparum* it was possible within 8 weeks of stopping suppressive treatment to establish whether cure had been attained. In over 100 volunteers inoculated with blood containing *P vivax* or *P falciparum* no instance of insusceptibility was found. Similarly in experimental mosquito-transmitted malaria in fifty controls not receiving antimalarial drugs, only two failed to develop overt malaria. One had been bitten by two mosquitoes proved by subsequent dissection to contain sporozoites of *P vivax* in the salivary glands. The other was bitten by six mosquitoes of a 60 per cent. infected hatch (*P falciparum*) Both volunteers were subsequently re-exposed to eleven infective bites and developed overt attacks of B.T and M.T malaria respectively within the usual incubation period.

In view of the ideal conditions under which these large scale experiments were carried out, and the resulting 100 per cent. transmission achieved it would appear that the European who had not previously been exposed to malaria is never refractory to infection provided viable sporozoites or trophozoites of *P vivax* or *P falciparum* be inoculated in a reasonable dosage In the white man of European ancestry natural immunity to jungle malaria is a myth—at least as far as New Guinea strains are concerned

(C) SUBINOCULATIONS IN CONTROLS AND VOLUNTEERS TAKING ANTI MALARIA DRUGS.

The modern view on the biology of the malaria parasite in the vertebrate host is that sporozoites introduced into the body pass via the blood stream to endothelial cells within which they undergo development, after which there is a discharge of parasites into the blood stream. In contrast to most of the erythrocytic parasites exoerythrocytic forms are non-pigmented. Though encountered in several species of bird malaria the tissue forms (cryptozoites metacryptozoites, exoerythrocytic forms etc) have never with certainty been demonstrated in man.

Subinoculations with blood from malaria patients have been common practice Generally however not more than 10 c.c. of blood containing malaria parasites have been injected. In order to investigate the presence of blood parasites where thick films were negative it was decided to use 200 c.c. of blood as a routine (approximately 4 per cent. blood volume) In some instances as much as 500 to 800 c.c have been used.

The results obtained by subinoculation at different times in volunteers

experimentally infected with *P. vivax* or *P. falciparum* may be summarised
as follows —

(1) Seven minutes after being bitten on one arm by anophelines (*A. punctulatus var. typicus*) infected with either *P. vivax* or *P. falciparum* direct blood transfusion has the other arm has produced malaria in the recipient, showing that sporozoites were circulating at that time. In one case a direct transfusion with 500 c.c. of blood made at the actual time of biting yielded a positive result. After 30 minutes subinoculations were negative, but in one instance a volunteer developed a persistent enlargement of the spleen and a palpable liver unassociated with demonstrable parasites or primary malarial fever. The case, which is of considerable interest, is still under observation.

(2) In *P. falciparum* infections, blood (200 c.c.) collected during the first 8 days, failed to transmit malaria, but subinoculations yielded positive results from the 7th day after exposure onwards. Parasites may not be demonstrable in thick smears for from 1 to 3 days after the blood is first proved to be capable of transmitting malaria by subinoculation, i.e., 7th day.

(3) In *P. vivax* infections direct transfusion of 200 c.c. of blood during the first 8 days failed to transmit malaria, but on the 9th day subinoculations were invariably positive. Thus, in our series ten out of ten subinoculations of 200 c.c. of blood from *P. vivax* infections were negative on the 8th day and fifteen out of fifteen were positive on the 9th day following infective bites (*P. vivax*)

(4) In *P. vivax* infections, however prolongation of the period of negative subinoculations can be obtained by drugs like plasmoquine, which evidently inhibit the intracellular development of the sporozoite and early tissue forms (cryptozoites and metacryptozoites). If given in large dosage (0·3 gramme daily) prior to, on the day of exposure to infection and for the next 5 days, negative subinoculations may be obtained up to the 10th day or later the incubation period is prolonged and overt malaria may not appear for 18 to 21 days

(5) When volunteers are taking atebrin in a dosage of 0·1 gramme daily we have found that subinoculations in *P. falciparum* are positive with blood collected on the 7th, 8th and 9th day even though parasites cannot be demonstrated in thick smears by the 11th or 12th day negative results are always obtained. Furthermore, if atebrin medication be continued in the dosage in *P. falciparum* infections the blood never regains its power of infectivity and cure is attained, whereas in *P. vivax* infections a negative subinoculation may later be followed by an overt attack of B.T malaria once atebrin suppressive treatment (0·1 gramme daily) has stopped, even though this treatment be prolonged for months.

The reappearance of erythrocytic forms in *P. vivax* after the blood has been completely cleared of parasites, no less than the tendency of benign tertian infections to relapse repeatedly despite prolonged anti-malarial treatment, suggests the persistence of a tissue stage (exoerythrocytic form) which from time to time throws off asexual parasites into the circulation for invasion of the erythrocytes. In M.T malaria the fact that the disease is readily cured and that erythrocytic forms do occur (as demonstrated by subinoculation) but are rapidly and permanently eradicated when the individual is taking 0·1 gramme of atebrin daily suggests that either the tissue phase in *P. falciparum* is naturally of short duration or that atebrin destroys the secondary tissue stages (exoerythrocytic forms) as well as the asexual parasites. The tissue stage only lasts a short time, this possibly explains why in a fatal disease like M.T malaria in which autopsies are possible, exoerythrocytic forms have never been demonstrated.

If the tissue cycle be 48 hours the sharp demarcation between negative

and positive subinoculation results in M T. and B T. respectively suggests that there are four cycles in *P. vivax* and three in *P. falciparum* before the metacryptozoites liberate erythrocytic parasites into the circulation.

The results of these subinoculation tests also suggest that when investigating —

(1) Sporozoiticidal action—the peak concentration of the drug should be obtained in the blood at the time of biting and for 30 minutes thereafter

(2) Action on the early tissue stages—the drug should be administered for the first 6 days in *P. falciparum* and for the first 8 days in *P. vivax* infections.

(3) Schizonticidal action—the drug should be administered from the 7th day onward in *P. falciparum* and from the 9th day onward in *P. vivax* these being the respective times at which erythrocytic forms are first demonstrable on subinoculation.

II Value of Sulphonamides in Preventing or Suppressing Experimentally Induced Malaria.

The drugs tested in this group were sulphadiazine, sulphamerazine and sulphamezathine When given in a daily dosage of 1 0 gramme sulphadiazine proved slightly more effective in suppressing and curing M T malaria than the others, but the series is so small that the differences noted are of doubtful significance. For present purposes the results with these drugs will be considered together

(A) blood levels.

Many estimations by Fantl's modification of Werner's method were made on the blood levels of the free drug 2 to 4 hours after administration and at the end of the 24-hour period in forty-five cases These were taken as representing the maximal and minimal blood levels attained by a daily dosage of 1 0 gramme of the drug The average mean minimum and mean maximum values with sulphamerazine were 3 3 and 5·6 mg per cent. with sulphamezathine 1·9 and 3 7 mg per cent. and with sulphadiazine 2 6 and 4 7 mg per cent. respectively It will be seen that the highest and best maintained concentration was obtained with sulphamerazine and the lowest with sulphamezathine sulphadiazine holding an intermediary place. Sulphamerazine constantly showed a higher and better maintained blood level and for this reason and owing to the rarity of nausea, mental depression and toxic complications it was ultimately selected for extensive investigations.

(B) malignant tertian malaria.

1 *Mosquito-transmitted Malaria*

Volunteers were exposed to ten infective bites (*P. falciparum*) over 7 days and received 1 gramme daily of one or other of the three drugs. Others acted as controls and received no drug The controls were exposed to infective bites under similar conditions and invariably developed overt malaria within the usual incubation period. Out of a total of twenty-one volunteers taking

sulphonamides one had an attack of overt malaria when taking the drug, while in the remaining twenty the infection was suppressed. In three of these, overt malaria developed 3, 9 and 11 days after drug suppressive treatment ceased. The remaining seventeen were ambulatory throughout the whole period of exposure to infection during the next 23 days while on drug treatment and for the next 5 weeks. Minor symptoms, including headache malaise and abdominal discomfort were noted on a few occasions during the period and four had transient demonstrable hepatomegaly. In three instances isolated parasites were demonstrated, on one day only in thick smears. At the end of the 5-week period a subinoculation of 200 c.c. of blood from each of the seventeen volunteers was made intravenously into another non-immune volunteer. The seventeen recipients all failed to develop malaria. Subsequently the original volunteers were inoculated intramuscularly with blood containing an estimated number of parasites (*P. falciparum*). In every instance overt M.T. malaria developed and *P. falciparum* was found in their blood; this showed they were susceptible and possessed no effective premunity to the strain of *P. falciparum* injected.

Summarizing the results it may be stated that the sulpha drugs used in these experiments effectively suppressed symptoms in twenty out of twenty-one infections and actually cured seventeen out of twenty-one (81 per cent.) volunteers exposed to M.T. malaria when given in 1·0 gramme dose during the period of exposure and throughout the incubation period of M.T. malaria, which was estimated at 23 days.

2. Blood-inoculated Malaria.

In the early stages of this investigation the value of subinoculation for demonstrating blood infections on the 7th, 8th and 9th day following *P. falciparum* infection had not been worked out. The evidence available suggested that the sulphonamides were acting as schizonticides rather than true causal prophylactics. To get further data it was decided to investigate the action of these drugs in trophozoite induced malaria using citrated blood containing an estimated number of parasites (*P. falciparum*).

In this experiment twenty-four out of thirty volunteers received 1 gramme of one of the sulphonamides under discussion the day before the intramuscular injection of blood containing an estimated number of parasites (*P. falciparum*) and daily for 23 days thereafter. The remaining six received the infected blood, but no drug and acted as controls. The controls all developed overt malignant tertian malaria within the usual incubation period, showing the potency of the blood in regard to malaria transmission.

Results.—In only four instances did volunteers develop frank clinical malaria and parasites; two while taking the drug and two shortly after drug administration ceased. In the remaining twenty volunteers symptoms were either entirely suppressed or were out of sufficient severity to necessitate the patient lying up during the period of 23 days throughout which the drug was being

administered and for 5 weeks thereafter At this stage subinoculations (200 c.c. of blood intravenously injected) into another group of twenty volunteers failed to induce malaria, showing that blood parasites were not present. Subsequent inoculation of the twenty original volunteers with blood containing M T parasites induced frank malaria in every instance showing that they were not naturally immune to M T malaria and had not developed an effective pre munity to the strain of *P falciparum* inoculated.

3 Comment

It is evident from these observations that in M T malaria these sulpha drugs are schizonticidal in action provided they are given over a sufficiently prolonged period It also appears highly probable that their prophylactic action in mosquito-transmitted malaria in man is also due to schizonticidal action rather than their lethal effects on sporozoites, for in three volunteers with suppressed malaria, in whom scanty parasites were demonstrated during the period of drug administration subinoculation of non immune volunteers at a later date failed to produce evidence of latent malaria. In these three cases, at least, more prolonged administration of the drug in the same dosage i.e., 1 0 gramme daily had resulted in disappearance of the asexual parasites and final cure.

(C) BENIGN TERTIAN INFECTIONS.

1 Mosquito-transmitted Malaria

In a similar type of experiment volunteers were exposed to mosquitoes infected with *P vivax* (twenty one to twenty three infective bites over 7 days) and given 1 gramme of one or other of the three sulpha drugs daily for 1 to 2 days before exposure, during the period of exposure and for 23 days after the last bite The controls invariably developed overt B T malaria within the usual incubation period. Twenty-one of the twenty four volunteers also developed overt malaria while taking the drug   in the remaining three volunteers malaria was suppressed during the period of drug administration, but an overt attack followed shortly after cessation of drug administration.

Another group of four volunteers were *lightly* infected, each receiving one infective bite on two alternate days (*P vivax*) from mosquitoes which were subsequently dissected to show sporozoite infection in the salivary glands The value of a daily dose of 1 0 gramme of sulphamerazine administered as above was tested. In one volunteer malaria broke through during drug adminis-tration in two others symptoms were suppressed during this period, but malaria fever developed soon afterwards with parasites in the blood smears The fourth was a presumptive cure, for though one parasite had been seen on the 19th day subinoculation of 200 c.c. of his blood collected on the 66th day failed to produce malaria in another volunteer following an injection on the 70th day of 10 c.c. of blood containing parasites (*P vivax*) be himself developed typical B T malaria.

2. Blood-inoculated Malaria

In another group of volunteers the suppressive action of these three sulpha drugs was tested in twenty four volunteers infected with blood containing a calculated number of parasites (P. vivax). Each member of the group took I gramme daily on the day before inoculation and for twenty three days thereafter. The six controls who did not receive any anti-malarial drug all developed B.T. malaria within the usual incubation period.

Of the twenty four test volunteers twenty-one developed overt malaria with parasites in the blood during drug administration, while the remaining three volunteers, all of whom were taking sulphamerazine, developed overt malaria a variable time after cessation of the drug.

As in sporozoite-transmitted malaria, these sulpha drugs showed minimal suppressive action in trophozoite-transmitted malaria, and in no case was cure established in heavy infections with P. vivax.

3. Comment.

It is evident from these findings that these sulpha drugs, even in a dosage of 1 gramme daily could have only a very limited suppressive value in areas of malaria where P. vivax infections were prevalent. On the other hand, in places like West Africa where P. falciparum predominates and P. vivax infections are relatively rare, their chemotherapeutic value in the suppression and cure of malaria would be considerable, being probably superior to quinine, though definitely inferior to atebrin, as will be shown in a later section.

III. VALUE OF ATEBRIN AND OF ATEBRIN AND SULPHA DRUGS IN PREVENTING OR SUPPRESSING EXPERIMENTALLY INDUCED MALARIA IN MEN

Similar experiments were undertaken in groups of volunteers receiving 0·6 gramme or 0·7 gramme atebrin weekly and in another receiving 0·6 gramme atebrin weekly and 1·0 gramme sulphamerazine daily. In the case of atebrin, drug treatment was commenced 22 to 48 days and in the case of sulphamerazine 2 days before exposure to infection. These drugs were given under medical supervision throughout the period of exposure and for 23 days thereafter.

(A) MALIGNANT TERTIAN MALARIA.

1. Mosquito-transmitted Malaria.

10 volunteers received 10 infective bites during 4 seasons over 7 days

9	"	"	21	"	"	7	"	7
2	"	"	20	"	"	2	"	3
2	"	"	20	"	"	1	"	1
2	"	"	10	"	"	1	"	1

Nine controls were used in these groups and they invariably developed overt malaria and parasites within the normal incubation period of 11-12

malaria. Of the total twenty six volunteers comprising the three drug groups five took 0 6 gramme atebrin weekly thirteen took 0 7 gramme atebrin weekly and eight took 0·6 gramme atebrin weekly and 1 0 gramme sulphamerazine daily In all instances suppression was effective and judged by clinical criteria, subinoculation and other tests cure resulted.

Parasites —Every day over a period of approximately 29 days, 1 to 2 c.mm. of blood were examined for parasites ; subsequently routine thick films were examined daily Not a single trophozoite or gametocyte was seen in any of these cases either during or after drug treatment.

Clinical Features —Mild clinical features were noted in a number of cases These included headache, slight rise in temperature, malaise abdominal discomfort and tenderness over the liver and spleen in one case a palpable spleen developed which persisted on and off for 7 weeks

In no instance were symptoms sufficiently severe to necessitate bed rest, and volunteers invariably carried on their routine activities

Subinoculation. —Some 200 c.c. of blood, which was collected from twenty-two volunteers about the 65th day and from two other volunteers on the 44th day after the first exposure to infection were injected intravenously into twenty four non immune volunteers In not a single instance did malaria develop, indicating that none of the twenty four original volunteers were suffering from latent malaria (*P falciparum*)

Susceptibility Test —Approximately 7 days later sixteen of the original volunteers received an injection of 10 to 20 c.c. blood containing an estimated number of M T parasites into the gluteal muscles In every case typical malignant tertian malaria developed, showing that the individuals implicated were neither naturally immune to this species of malaria nor possessed effective premunity to the particular strain injected. In the remaining ten cases it was decided to test their susceptibility to sporozoites (mosquito-transmitted infection) instead of trophozoites (blood inoculation) In every instance following infective bites (*P falciparum*) the volunteers developed clinical malaria associated with asexual parasites in the blood followed by a gametocyte wave

2. *Comment*

Fifty volunteers were used in these experiments twenty-six received infective bites and none developed overt M T malaria the other twenty four received subinoculations with negative results When these experiments were originally devised it was not anticipated that 0·6 gramme atebrin ~~daily~~ would prove so effective in suppressing and curing malignant tertian malaria transmitted by experimentally infected mosquitoes. A combination of atebrin and sulphamerazine was used to ascertain if their combined action would be more effective than either drug singly Owing to the efficacy of the 0 6 gramme weekly dose however it proved impossible to demonstrate if synergic action could be attained in this manner

The absence of demonstrable parasites, the failure of subinoculation to

transmit malaria and the final demonstration of susceptibility and absence of premunity to the M T parasites injected in every volunteer constituted a chain of evidence indicating that malarial infection had either been prevented by these anti malarial drugs or cure attained

Early Subinoculation Tests—Subinoculation tests in six out of six volunteers taking 0·6 or 0·7 gramme atebrin weekly proved positive in all instances when 200 c c of blood were injected some 7 to 9 days after the exposure to infective bites (P falciparum) despite the fact that parasites were never demonstrable in thick blood smears at the time or at any subsequent stage of the experiment. From the 11th to 22nd day after exposure to infective bites (P falciparum) similar subinoculations in six out of six volunteers taking 0·6 to 0·7 gramme atebrin daily were negative and in all cases final cure was proved to have occurred. These findings indicate that in M T infections suppressive atebrin (0·6 and 0·7 gramme weekly) (1) does not act as a causal prophylactic (2) destroys the young asexual parasites as they enter the circulating blood from the 7th day onwards. The fact that parasites cannot be demonstrated by subinoculation later than the 10th day indicates that cure by 0·6 to 0·7 gramme weekly is attained during this limited period when young asexual parasites are emerging from the primary tissue stages passed in endothelial cells, and the group plasma atebrin averages 21·3 and 23·0 μg. per litre.

(B) BENIGN TERTIAN MALARIA

1 Mosquito-transmitted Malaria

Two groups of test cases and four controls were subjected to twenty-two to twenty three infective bites over a period of 1 week in daily sessions. In the four controls receiving no drug treatment and exposed under similar conditions all developed typical B T malaria parasites appeared from the 11th to 18th day and fever from the 12th to 18th day following first exposure to infection. Atebrin in a dosage of 0·6 gramme weekly was administered to five test cases over a period of 18 to 49 days prior to first exposure to infection, and for 23 days thereafter. In addition, the second group of seven cases received 1·0 gramme sulphamerazine 1 to 2 days prior to first exposure to infection throughout the period of infection and for 23 days thereafter. A third group of test cases and four controls were exposed to twenty infective bites in one session on 1 day. The test cases having atebrin 0·1 gramme daily for 7 days a week continued for the same period as the other two groups.

In no instance in any group did malaria break through during the period of drug administration but in every case clinical malaria associated with demonstrable parasites developed later. In these cases malaria fever appeared from 14 to 44 days, and parasites from 19 to 46 days after drug treatment ceased.

No parasites were found in thick films during drug administration but mild clinical features were noted in three cases in each group during this period. They included transient mild fever headache and abdominal discomfort

slight tenderness and enlargement of the liver or spleen were noted in a few instances In no case was the clinical syndrome of sufficient severity to make the patient take to bed or modify his routine daily activities

2 Blood-inoculated Malaria

Three groups comprising four volunteers each were investigated In each group one volunteer who received no anti malarial drugs acted as a control The other three received drug treatment consisting of atebrin (0·6 gramme weekly) with or without sulphamerazine or sulphamezathine (1·0 gramme daily) The first two groups received 15 c.c of blood containing 225 million parasites intramuscularly the third group received 20 c.c of blood con taining 182 million parasites All three controls developed malaria fever parasites being demonstrated from the 16th to 21st day

In no cases did overt malaria develop either while on the drug or during the subsequent 5 to 6 weeks observation which followed. Subinoculation with 200 c c of blood into nine fresh volunteers followed from the 58th to the 64th day the results were uniformly negative, none developing malaria.

Susceptibility tests followed, the original nine volunteers receiving 10 to 20 c.c of blood containing an estimated number of B T parasites Malaria fever resulted parasites being demonstrated from the 8th to 11th day in all instances

These results clearly showed that not only was suppression complete but that every volunteer in the three groups was cured by the action of these anti malarial drugs on the asexual B T parasites (New Guinea strain) The readiness with which trophozoite inoculated B T malaria is cured stands in marked contrast to the difficulty experienced in curing sporozoite induced B T infections in the South West Pacific Area and as JAMES and others have suggested, supports the view that exoerythrocytic forms of P vivax are produced in sporozoite infections which are resistant to drug therapy

(C) CONCENTRATION OF ATEBRIN AND SULPHAMERAZINE IN THE BLOOD

In a combined series of observations on thirty-one individuals experimentally infected with mosquito-transmitted malaria (M T nineteen cases B T twelve cases) ninety one atebrin estimations were made The average mean of the group taking 0·6 gramme atebrin weekly was 21 3 μg per litre the absolute minimum and maximum variations being 0 to 46 μg per litre The average mean of the group taking 0·6 gramme atebrin and 7 0 grammes sulpha merazine weekly was 23 1 μg per litre the absolute minimum and maximum variation being 7·0 to 48 μg per litre The average mean of the group taking 0 7 gramme atebrin weekly was 23·0 μg per litre the absolute minimum and maximum variations being 10·0 to 35 μg per litre.

Estimations by Fautl s modification of Werner's method on the sulpha merazine content of whole blood were made before the drug was administered

and 4 hours later   The average mean prior to daily drug administration was 3 78 mg per 100 c.c., the mean of the minimum readings being 3 28 mg and the mean of the maximum readings 4 19 mg   Estimations 4 hours after administration of the drug showed an average mean of 5·67 mg per 100 c.c., the mean of the minimum readings being 5 21 and the mean of the maximum readings being 6 29 mg per 100 c.c.   Considering this dosage of 1·0 gramme was administered only once in the 24 hours   the concentrations observed were eminently satisfactory and definitely greater than those obtained in a series of observations in volunteers receiving a similar dosage of sulphadiazine and sulphamerathine

IV   FIELD TYPE OF EXPERIMENT

VALUE OF SUPPRESSIVE ANTI MALARIA DRUG TREATMENT IN VOLUNTEERS BITTEN REPEATEDLY OVER A PERIOD OF SEVERAL MONTHS BY ANOPHELINES INFECTED WITH P FALCIPARUM AND P VIVAX

Preceding experiments dealt with the value of certain sulpha drugs and atebrin from the standpoint of causal prophylaxis and suppressive drug treatment in volunteers who had been infected with either P falciparum or P vivax for a period not exceeding 1 week.

These conditions differed from those existing in jungle fighting in hyper endemic areas of malaria where troops are liable to be bitten over a prolonged period and infected with both species of parasite.   Under such circumstances it has been universal experience that troops generally break down with malignant tertian malaria and that only parasites of P falciparum are found in their smears of the blood in most instances.   Later after conditions become more acute, or when troops return to the mainland of Australia and cease taking suppressive atebrin they relapse predominantly with malaria of benign tertian type, P vivax being found in blood smears.

It might be thought that this phenomenon was dependent on suppressive drug treatment and undoubtedly if it is improperly carried out, this is one of the factors implicated.   Results obtained in this experimental investigation however   indicate that if the daily 0·1 gramme dose of atebrin has been taken, neither M T or B T malaria should break through during the period of atebrin administration, and that if atebrin be taken regularly for 4 weeks after the last exposure to infection   M T malaria should have been cured, whereas B T malaria develops with great regularity later   Furthermore troops who develop malaria fever in jungle warfare are admitted to hospital and receive a thorough course of treatment which is curative for the malignant tertian infection in the vast majority of instances, leaving the latent benign tertian malaria to relapse later

Apart from these considerations, however the present investigation has shown that if an untreated volunteer be infected on the same day by the same number of anophelines harbouring sporozoites of P falciparum and P vivax, the resulting primary fever is of malignant tertian type and parasites of P falci-

parum are found in thick smears, the benign tertian infection remaining latent. As a rule once parasites of *P falciparum* appear in demonstrable numbers in the blood their rate of increase is much more rapid than that of *P vivax*, and when the two species are in competition it is malignant tertian malaria which breaks through apart altogether from considerations of drug therapy

Furthermore, it has been found in volunteers repeatedly infected with B T and M T parasites who are taking smaller doses of atebrin (0 3 to 0·4 gramme weekly) that benign tertian infections remain suppressed whereas malignant tertian malaria breaks through with great regularity

Though many of the experiments already described were undertaken in the humid tropics at the hottest time of the year they at least differed from those obtaining in jungle warfare, inasmuch as the volunteers were not subjected to those mental stresses physical strains and exhaustion, inadequate dietary, dehydration blood loss, etc. which troops fighting in the jungle might and often do experience. In the later groups of volunteers efforts have been made to introduce such factors as are regarded as favouring the precipitation of relapses in cases of latent malaria. These will be discussed later

DETAILS OF EXPERIMENT

1 *Drug Administration*

Observations were made in two series of volunteers the first containing thirty men and the second twenty-five men that is fifty-five in all These series were divided into groups of six men as a rule two being placed in each of the following regimens 0 1 gramme atebrin daily 0 1 gramme atebrin and 1·0 gramme sulphamerazine daily and 0 2 gramme atebrin daily Table I shows the men placed in each regimen —

TABLE I

VOLUNTEERS OBSERVED ON EACH DRUG REGIMEN

Drug Regimen	Number of Volunteers.		Totals.
	Series I	Series II	
0 1 gramme atebrin daily	10	15	25
0 1 gramme atebrin and 1·0 gramme sulphamerazine daily	10	4	14
0 gramme atebrin daily	10	6	16

Drug administration commenced 24 to 110 days before exposure to infection in the case of atebrin and for 2 to 3 days in the case of sulphamerazine it was continued throughout the period of exposure (approximately 3 months)

and for 28 to 34 days after the last bite. Four men had an atebrin "build up" of 0·4 gramme daily for 3 days before first exposure to infection.

2. Exposure to Infection

Anophelines used in these experiments were mainly *A. punctulatus* or *typhus*. Sporozoite infections of the salivary glands were as a rule heavier than would be anticipated in nature as only carriers showing a high gametocyte count in the blood were selected to produce infection; daily dissections of the salivary glands were made to control this factor.

The number of infective bites was also large. As patients were continually under skilled medical observation, it was considered legitimate to err on the side of excessive infection in a critical experiment designed to assess the effectiveness of anti-malarial drugs in hyperendemic areas of malaria, where from time to time personal protective measures might be non-existent or frankly inadequate.

The number of infective bites varied from two to thirty five per session. There were ten to twenty sessions over the period of exposure which ranged from 49 to 92 days. Table II sets out the detail of the infections in these

TABLE II

INFECTIVE BITES FOR VOLUNTEERS IN LONG-TERM EXPERIMENTS.

Number of Volunteers in Group	Series	Group	Infective Bites				Totals	Number of Biting Sessions
			Ranges		Average			
			P. falciparum	P. vivax	P. falciparum	P. vivax		
6	I	A	4...4	19-..	4	20	62	13 over 82 days
6		B	30-3*	18-~0	21	19	50	13 over 89 days
6		C	37-35	18-18	22	16	48	13 over 89 days
6		D	37-40	1-.17	22	16	54	11 over 71 days
6		E	59-8*	3-~37	90	36	96	11 over 89 days
6	II	F	90-94	35-37	91	36	177	14 over 6* days
6		G	73-74	40-4	4	41	115	14 over 68 days
4		XVI A	41-43	29-31	4.	30	7*	10 over 68 days
6		XVI B	8 90	36-3*	89	28	1.5	11 over 73 days
3		VI C	101 117	9 -83	105	82	190	11 over 74 days
3		VI B	145-15.	100-107	150	} 103	253	11 over 69 days
3		VI C	165-157	~8-78	156	~8	234	17 over 69 days
Totals 53			30-157	18-107			49-33	10-20 over 49-92 days

volunteers. It will be noted that the general practice has been to expose to
P falciparum and P vivax in a ratio of 2 : 1

For each new batch of mosquitoes an untreated volunteer acted as a control
and received the same number of infective bites as the members of the groups
taking suppressive drugs Twenty-six of the twenty seven controls used
developed B T or M.T malaria within the normal incubation period. The
one control who failed to develop malaria had received three (calculated)
infective bites (batch of A punctulatus var typicus mosquitoes—60 per cent.
infected with P falciparum) He was subsequently re-exposed to eleven
infective bites and developed M T malaria within the usual incubation period
showing that he was not naturally immune

3 Parasites

Prolonged search for malaria parasites was made throughout the period
of experiment in every volunteer using modified Field's staining of thick
smears. For the first 7 weeks approximately 0 2 c.mm. of blood were examined
every day and for the subsequent 5 weeks every 2nd day and at any additional
time thought advisable Subsequently ordinary thick smears were searched
without measuring accurately the quantity of blood examined.

Parasites were seen in only four out of fifty-five volunteers throughout
the whole period of observation which covered drug treatment (3 to 4 months)

(a) One volunteer receiving 0 1 gramme atebrin and 1·0 sulphamerazine
daily showed less than 1 parasite per c.mm. (considered to be P vivax) on the
41st and 42nd day at a time when the blood contained 10 μg per litre of
atebrin.

(b) One volunteer receiving 0 1 gramme atebrin daily showed one parasite
in 3 c.mm. of blood, considered to be P falciparum on the 62nd day His
plasma atebrin level at this time was 12 μg per litre.

(c) One volunteer taking 0 2 gramme atebrin showed one ring form con-
sidered to be P falciparum on the 23rd day after exposure the next day the
blood atebrin equalled 74·0 μg per litre.

(d) The fourth volunteer who was in the 0 2 gramme atebrin group showed
parasites, P falciparum from the 98th to 105th day the counts being 350 140
560 and 6 per c.mm. For some time it had been noted that this man had
become less yellow and that the blood atebrin values were falling progressively
until the low level of 7 μg per litre was attained during the period of parasit-
aemia. He was closely questioned and following this the atebrin blood level
progressively rose to 50 μg per litre parasites in the meantime disappearing
without clinical malaria developing Independent evidence was obtained that
this patient had been surreptitiously avoiding taking atebrin. In assessing the
clinical features and plasma atebrin levels which developed in these groups
this case will not be included as it is known with certainty that his atebrin
intake was unsatisfactory (Graph 1 page 332).

A fifth volunteer (Graph 2) a non-co-operator was also excluded from this series. His plasma atebrin level fell from 37 μg. per litre on the 42nd day to 0·0 μg. on the 70th day at which time M T parasites were found in the blood and fever occurred though not sufficiently severe to necessitate his going to bed. He was closely questioned about avoiding taking atebrin and thereafter parasites and fever rapidly disappeared and within a week the atebrin plasma level rose to 32 μg. per litre. Later he absented himself without leave and owing to the risk incurred by such conduct he was immediately given a full course of treatment for malaria and subsequently discharged to his unit. Evidence from other volunteers confirmed that this man had been avoiding

FALL IN ATEBRIN LEVELS ON AVOIDING DAILY DOSE.
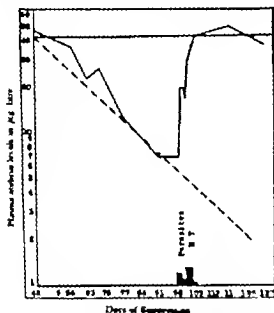
GRAPH 1—Volunteer (3·4 gramme week regime).
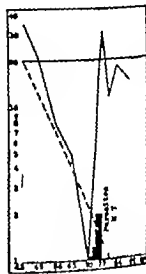
GRAPH 2.—Volunteer (0·7 gramme week regime).

Mean atebrin level for this regime and "die away" shown for comparison.

taking atebrin in the prescribed dosage of 0·1 gramme daily. This is generally accomplished either by palming the tablet or by retention in chewing gum into which the tablet can be pushed by the tongue and subsequently recovered

4 Clinical Features

A break through was regarded as having occurred if there was a temperature over 100 F parasites were present in the blood, and the patient was so ill that he had to take to bed. Such an attack is of sufficient severity to necessitate evacuation to hospital. No break through occurred in any of the 55 test cases. Their general health remained excellent. Three men were confined to bed

for upper respiratory tract infections for 2 to 3 days It is estimated that the total time lost would not have amounted to more than 21 out of 4,260 man days (0·5 per cent.) this included the three men mentioned above and another who had a chronic antral infection. A temperature over 100 F associated with minor toxic symptoms such as headache or backache was observed in fifteen, but this was invariably of transient duration. During this investigation it has been frequently noted that in the tropics volunteers occasionally develop a temperature lasting a few hours before they have been exposed to malaria this may be related to heavy exercise in the heat of the day

The occurrence of minor clinical features is set out in Table III.

TABLE III

CLINICAL FEATURES OBSERVED IN FIFTY FIVE VOLUNTEERS REPEATEDLY EXPOSED TO MOSQUITO-TRANSMITTED B T AND M.T MALARIA WHILE TAKING SUPPRESSIVE DRUG TREATMENT

Drug Group	Number in Group	Temperature to 100 F	Number with palpable spleen	Number with palpable liver
Atebrin 0 1 gramme/day	25	10	3	7
Atebrin 0 1 and sulpha merazine 1·0 gramme/day	14	1	1	4
Atebrin 0 2 gramme/day	16	4	1	2

The weight was generally well maintained and no significant decreases were observed. There was a slight fall in the red cell and haemoglobin content of the blood. This might be attributed to latent malaria, but might equally be due to 4 months residence in the humid tropics and altered conditions of life The actual findings are summarized in Table IV

TABLE IV

RED BLOOD CELL COUNTS AND HAEMOGLOBIN LEVELS IN VOLUNTEERS REPEATEDLY EXPOSED TO MOSQUITO-TRANSMITTED B.T OR M T MALARIA.

Drug regimen in gramme per day	Number of volunteers	Red blood cells Millions per c.mm.		Haemoglobin in grammes per 100 c.c	
		Before Exposure	After last Exposure	Before Exposure	After last Exposure
Atebrin 0 1 gramme	25	5 33	4 93	15 8	15·0
Atebrin 0·1 gramme sulphamerazine 1·0 gramme	14	5 16	4 0..	16 4	14·9
Atebrin 0 2 gramme	16	5 09	4 93	15 9	14·6
Totals	55	5 23	4·90	16·0	14 9

It will be seen that the average decrease following repeated heavy exposure to malaria infection over a period of several months was 320,000 red blood cells per c.mm. and 1 1 gramme of haemoglobin per 100 c.c.

5 Atebrin Plasma Concentrations

The atebrin plasma concentrations were made from time to time throughout the period of the experiment, i.e. from period of exposure to cessation of drug (77 to 120 days). The means are arithmetic and geometric. The results are epitomised in Table V

TABLE V

ATEBRIN PLASMA LEVELS IN MICROGRAMMES PER LITRE, SERIES I AND II.

Groups	Atebrin 0·1 gramme daily	Atebrin 0·1 gramme and Sulphamezone 1·0 gramme daily	Atebrin 0·2 gramme daily
Number of men	5	14	11[*]
Total tests	43	134	163
Average minimum	12 8	11·0	27 3
Average maximum	38	37·4	81·6
Arithmetic mean atebrin level of groups in µg litre	22 0	22 3	44 2
Geometric mean atebrin level of groups in µg litre	22 3	17 7	4. 9

One case known to have been taking steps to avoid atebrin has been excluded.

The average mean of the group taking 0·1 gramme atebrin daily was 22·0 µg per litre, the absolute minimum and maximum variations being 7 to 90 µg per litre. The average mean for the group taking 0·1 gramme atebrin and 1·0 gramme sulphamezone was very similar the average mean being 22·3 µg per litre and the absolute minimum and maximum variations being 0·0† and 67 µg per litre.

In the group taking 0·2 gramme atebrin daily the average mean was 44·2 µg per litre, and the absolute minimum and maximum variations were 0† and 90 µg per litre. As far as a sustained high atebrin plasma level is concerned, a dosage of 0·2 gramme daily is definitely superior to 0·1 gramme daily.

† The zero readings were obtained on specimens of plasma that had to be sent several hundred miles before atebrin estimations could be made.

In all groups however, break throughs failed to occur despite repeated infection so it can be assumed that an average mean plasma level of 22·9 μg per litre is adequate to prevent the development of malaria fever in a group even when the individual plasma values are subject to considerable variation

Observations on the atebrin plasma levels on the day break through occurs after cessation of atebrin treatment are set out in Table VI

TABLE VI

RESULTS OF SUPPRESSIVE THERAPY IN VOLUNTEERS REPEATEDLY EXPOSED TO
MOSQUITO-TRANSMITTED B.T AND M T MALARIA.

Number of volunteers in group	Suppressive regimen. Grammes per week.	Number of volunteers with overt malaria.	Type of *Plasmodium*	Mean days after ceasing suppression to overt attack.	Mean plasma atebrin level on suppression to μg litre *	Mean plasma atebrin level at overt attack.	
						μg litre.	Number of readings.
25	Atebrin 0 7	25	*P vivax*	29 9 (14–58)	22 2	3 4	20
14	Atebrin 0 7 Sulpha merazine ·0	14	*P vivax*	29 1 (19–49)	1″ ″	3 2	1 4
15	Atebrin 1 4	15	*P vivax*	63·9 (33–96)	42 9	2 2	16

* μg = microgramme

The Plasma Atebrin Level attained by the Prolonged Administration of 0 1 gramme Atebrin daily
showing Build up Equilibrium Level and Die Away

Though the results of plasma atebrin estimations made in the different groups of test volunteers have been summarized in various experiments it would be advantageous at this stage to include a special study made by Lieut - Colonel C. BICKERTON BLACKBURN Lieutenant K C POPE, and other members of the L.H.Q. Medical Research Unit, of the plasma atebrin levels in thirty five volunteers taking 0 1 gramme atebrin daily for a prolonged period, in order to show the build up equilibrium level and die away

All estimations were made on plasma using the double extraction method of BRODIE and UDENFRIEND Plasma specimens were collected 16 to 24 hours

after the last dose in all except some 5 per cent. of the specimens. Volunteers were all exposed once or more to mosquitoes infected with *P cynex* and *P falciparum* the only exceptions being some of the readings on build up

All means are geometric unless otherwise specified and graphs are set out on semi-logarithmic paper to conform with the general treatment of results

A number of the earlier estimations were made by Lieutenant Bero and Lieutenant Trager, of the U S Army

Equilibrium Levels

(a) *Group means, plasma levels*

Table VII sets out the detail of the thirty five volunteers investigated It will be noted that these men were studied over varying periods, the observations being made over a total period of some 10 months in a tropical climate during summer and autumn.

The degrees of activity varied from resting conditions (in the military sense) to severe exertion.

None of these volunteers developed overt malaria during this phase of observation

It is apparent that there is a considerable variation in the individual means, and that the determining factor is not body weight. These men were observed under similar conditions and took their atebrin daily on a scale designed to ensure regular dosage
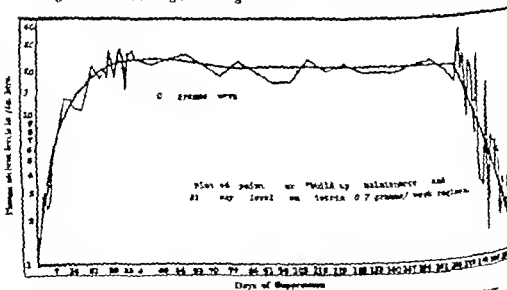
GRAPH 3 —MEAN PLASMA ATEBRIN LEVELS IN VOLUNTEERS TAKING 0·7 GRAMME ATEBRIN DAILY FOR 165 DAYS

Note "build up" maintenance level and "die away" following cessation of atebrin on 165 th

On many volunteers there were few observations as they were being used for short term experiments

TABLE VII

INDIVIDUAL MEAN ATEBRIN LEVEL IN THIRTY-FIVE VOLUNTEERS ON ATEBRIN 0 7 GRAMME PER WEEK REGIMEN

Dosage in mgm./kg./day	Number of readings.	Period of atebrin covered by readings (days).	Mean atebrin levels μg. per litre.	Range of atebrin levels. μg per litre.
1·55	2	46 – 61	25 70	23 – 35
1 39	8	44 – 85	13 60	9 – 23
1 38	2	46 – 61	23 50	20 – 27
1·45	2	46 – 61	18 40	17 – 20
1·61	19	73 – 158	24·04	15 – 36
1 33	–	52 – 127	23 10	17 – 37
1 72	2	46 – 61	23·00	33 – 33
1 11	3	46 – 61	29 20	25 – 32
1·61	3	46 – 61	20 50	17 – 23
1·60	10	57 – 155	20·021	10 – 30
1·41	2	50 – 61	24·00	23 – 25
1 39	10	44 – 134	19 20	13 – 33
1·62	8	45 – 10	31 40	17 – 47
1·99	8	45 – 107	33 30	20 – 53
1 29	7	43 – 85	17 70	12 – 22
1 72	–	43 – 85	20 80	12 – 14
1 21	11	45 – 127	18 45	5 – 36
1 51	–	43 – 106	21·90	11 – 31
1 16	8	47 – 113	27 90	23 – 37
1·45	14	43 – 88	20 65	15 – 34
1 59	15	43 – 88	17 70	12 – 22
1 13	4	44 – 53	29 10	15 – 38
1·67	10	96 – 197	11 12	3 – 39
1 73	10	44 – 134	20·60	70 – 43
1 87	13	18 – 78	23 77	12 – 26
1 78	6	64 – 92	23·07	12 – 23
1 12	12	65 – 149	16 11	9 – 22
1 59	4	44 – 53	22 10	17 – 23
1·64	11	45 – 127	24 21	16 – 33
1·60	10	43 – 112	32 50	18 – 67
1 45	9	43 – 112	22·00	7 – 3
1 19	10	96 – 197	28 90	19 – 57
1 50	12	65 – 149	18 71	5 – 30
1 51	8	47 – 113	18 40	7 – 37
1·60	10	110 – 208	24 42	10 – 90

Group of mean₀ atebrin level   22·5 μg per litre
Range of mean₀   11 1 to 33·0 μg per litre
Mean (Arithmetic) dose   1 51 mg per kg per day

Thirty five volunteers exposed to experimental mosquito-transmitted malaria have been specially investigated over a long period whilst taking stebrin 0 1 gramme daily for 7 days a week. The results obtained may be studied in Graph 3 page 336

The plasma stebrin levels between the 6th and 23rd weeks were as follows

(a) Group mean$_0$ plasma stebrin level   22 5 μg per litre.
     Range of means$_0$ 11 1 to 33·0 μg. per litre.

(b) Mean$_0$ plasma stebrin level   21·9 μg per litre.
     Standard deviation   1 56 μg per litre.
     Range of readings   7 to 90 μg per litre.

The equilibrium level was well maintained over a period of 17 weeks (6th to 23rd weeks)—no decrease in level was observed.

The mean$_0$ plasma stebrin level was reached at the end of the 4th week exceeded between the 5th and 11th weeks, and maintained at a constant level between the 11th and 23rd weeks of administration of stebrin 0·7 gramme per week.

On ceasing administration of stebrin the plasma levels showed a rapid diminution, zero readings being obtained about the end of the 6th week after the last dose was given.

The reported observation that there is a diminution of the equilibrium blood stebrin level in men on a constant regime over a considerable period of time may be due to the initial rise over the equilibrium level and subsequent return noted above.

(b) *Mean$_0$ plasma stebrin level*

Two hundred and eighty four readings were made on thirty-five volunteers the readings have been treated statistically and the following results obtained —

     Mean$_0$ plasma stebrin level   21·9 μg per litre.
     Standard deviation   1 56 μg per litre.
     Range of readings   7 to 90 μg per litre.

Maintenance of Level

Table VIII sets out the weekly means$_0$ observed on these volunteers from the 6th to 23rd weeks.

Graph 3 (page 336) shows these means$_0$ in relation to build up and decay. Observations from the 6th week to 23rd week have been selected as it has been stated that 6 weeks are required to establish the equilibrium for a given dosage regime.

It is apparent that there is no diminution of level in this period, the mean$_0$ plasma stebrin level of over 20 μg per litre is regarded as being quite adequate to suppress either benign or malignant malaria.

TABLE VIII

MAINTENANCE OF LEVEL.

Weeks on suppression.	Number of Readings.	Mean atebrin level μg per litre	Weeks on suppression.	Number of Readings	Mean atebrin level μg per litre
6	30	22 1	15	3"	23 8
7	16	23 0	16	13	20 0
8	12	26 1	1"	25	22 2
9	34	22 2	18	14	19 7
10	23	18 7	19	22	19 7
11	26	24 5	20	5	19 "
12	23	19 4	21	13	21 6
13	29	16 6	22	3	23 0
14	18	16 9	23	4	19 5

Build Up

Table IX sets out the daily means$_G$ obtained on some of the thirty-five volunteers used in these studies. Graph 3 shows these readings and the general curve drawn through them. While there is considerable daily variation in the levels little difficulty has been experienced in preparing the curve. The mean$_G$

TABLE IX.

BUILD UP IN ATEBRIN 0 7 GRAMME PER WEEK REGIMEN

Days since beginning atebrin.	Number of readings	Mean atebrin levels. μg. per litre	Days since beginning atebrin.	Number of readings	Mean atebrin levels. μg. per litre.
1	4	1 90	25	4	18 6
2	4	1 80	26	7	17 4
3	4	3 0	28	8	20 7
4	4	2 3	29	4	1" "
5	4	2 6	30	6	28 2
6	4	4 4	32	10	1" 5
	4	5 0	33	7	23 6
1.	2	12 8	34	3	27 9
14	2	12 8	35	5	24 7
16	2	11 2	36	3	14 8
19	2	10 9	37	13	26 1
21	2	15 5	38	5	26 1
23	2	21 4	39	4	27 8
			40	2	23 9

level is reached in 28 days and does not return to it till the 11th to 12th week
—the highest point of the curve being nearly 26 μg per litre at the 5th to 6th
week. This rise over the equilibrium with subsequent return to a constant
level of 20 to 21 μg per litre may explain the reported diminution in level of
blood atebrin observed on a constant regime of atebrin 0·6 or 0·7 gramme
per week. It is felt that this diminution is unimportant so long as it is appreciated
that the true equilibrium level is not reached for some 12 weeks after
commencing a constant regimen of 0·7 gramme per week.

Die Away

Table X sets out the daily means observed and Graph 3 shows the
curve plotted through these readings.

The die away approximates a daily decrease of 10 per cent. of the level
present. Zero readings are obtained at about the end of the 6th week after
ceasing to take atebrin.

Considerable variation in readings was obtained during the die away
period, this becoming more marked with the increase in time and consequent
lowering of plasma concentrations. It is well recognised that the method
employed has minimal accuracy when plasma atebrin concentrations are less
than 10 μg per litre.

TABLE X.

DIE AWAY AFTER CEASING 0·7 GRAMME PER WEEK REGIMEN.

Days since ceasing atebrin.	Number of readings.	Mean atebrin levels μg per litre	Days since ceasing atebrin.	Number of readings.	Mean atebrin levels. μg per litre
1	5	16·0	19	3	1·1
4	4	39·8	20	3	4·0
4	5	20·6	21	2	4·1
5	5	3·0	22	1	0·0
6		1·0	23	6	3·5
		16	24	4	4·75
			25	7	3·5
9		20·5	26	2	4·0
10	4	1	27	1	2·1
11	11	8	28		3·1
1		28	30	5	3·5
13		4	31		3·6
14	9	5	32		13·0
16	5	2·1	34	1	1·0
17	2	12·1	35	1	1·0
18	6	6	39	1	1·6

6 *Investigation of Factors that possibly Induce Malaria break throughs*

Volunteers in Series I Groups A B, C and D had considerable exercise which included walking swimming football and cricket, but only in Group E were the men subject to heavy exercise such as wood chopping, and walking over the hills through the hottest time of the day in tropical heat. In Series II, it was decided to —

(1) infect these volunteers more heavily than in Series I

(2) institute really heavy exercise throughout the day in a tropical humid climate

(3) investigate the effects of adrenalin insulin, cold and anoxia in members of the different groups

Adrenalin —Under conditions of jungle fighting psychical stress must frequently lead to stimulation of the suprarenal glands and to considerable excess of circulating adrenalin. As adrenalin injections are reported to induce parasitaemia in latent malaria by causing contraction of the spleen it was decided to inject adrenalin into volunteers receiving these suppressive drugs and to observe the effect on parasitaemia and clinical relapse. In cases so investigated to date, the results have been entirely negative. Twelve of the twenty five members of Series II received multiple injections of adrenalin on one or more occasions. They were given several doses of 0 5 c.c. of 1 in 1 000 solution—either in hourly doses for 4 hours or 2-hourly for six doses. No effects other than some shakiness and transient tachycardia were noted and malaria parasites never were demonstrated.

Insulin injections —Similarly it was considered that where troops were fighting periods might occur when the blood sugar would be considerably reduced. Injections of insulin were given to eighteen of this series in dosage up to 25 units once or twice a day. Sometimes insulin would be given twice a day for a week. Blood sugar levels from 40 to 70 mg per 100 c.c. were obtained without leading to the appearance of parasites in the blood or overt malaria.

Chill —The effects of chill were investigated by placing volunteers in a refrigeration chamber at − 9° C for 1 hour clothed in boots and trousers only all movement being restricted. In sixteen volunteers so exposed, no effect in inducing malaria break through or parasites was observed

Fatigue —Though the effects of fatigue were not so pronounced as in prolonged jungle fighting these volunteers were worked and exercised in a tropical climate at the hottest time of the year to a point verging on physical exhaustion. Some chopped wood throughout the day for 5 days of the week. Others were taken over hills for 6 to 10 miles, induced to swim against stream until they were tired out, and were then walked back over the hills at as fast a pace as possible by a specially trained sergeant major who was in charge of these groups when on exercise. More recently groups have been marched over a distance of from 80 to 85 miles in 3 days in mountainous country to a height

of 2,500 ft. blankets were not provided and despite the tropical latitude the nights were often cold.

Anoxia.—It is well recognized that anoxia possibly through contraction of the spleen tends to precipitate relapse in individuals with latent malaria.

To demonstrate the effects of anoxia 18 infected volunteers in Series II were flown from Cairns to Melbourne—a distance of approximately 2,000 miles, to be tested in the experimental decompression chamber at the Melbourne University by No. 1 Flying Personnel Research Unit. They were rested for 5 days at a military hospital in Melbourne where the weather was distinctly cold. They were then divided into two groups and spent approximately 1½ hours daily at 15,000 ft. without oxygen at 65 F. for 5 days. They were again rested for 7 days after which altitude runs at from 15,000 to 19,000 ft., were carried out at 28 F. Overcoats were worn. The time of exposure in the first 4 days was approximately 2 hours. On the 5th day in the first group a Bends run was made at 35,000 ft. at 65 F. with oxygen. the second group were given an anoxia run at 28° F. at heights varying from 15,000 to 19,000 ft., covering a period of 1½ hours. Careful clinical and laboratory investigations were made throughout the period covered by these experiments, but, despite repeated and prolonged examination of thick blood films, parasites were never demonstrable and no overt attacks of malaria occurred. The general conclusions reached were as follows:—

    (1) Anoxia and cold such as is likely to be encountered in air transportation of troops in the Pacific theatre of operations will not cause a break through in malaria-infected patients taking 0.1 gramme atebrin daily.

    (2) Observation of the men failed to reveal any significant difference in their anoxia responses which might be attributed to different doses of drugs used, i.e. 0.1 gramme atebrin, 0.2 gramme atebrin, and 0.1 gramme atebrin and 1.0 gramme sulphamerazine daily.

Subsequently these volunteers were flown back to Cairns. Investigations on arrival showed they were apyrexial, physically fit and without demonstrable malaria parasites in the blood.

7 Final Results of Suppressive Treatment

The final result of suppressive atebrin treatment was shown in Section II to be the effective suppression of both B.T. and M.T. fever and ultimate cure of M.T. infections, provided the drug (0.6 to 0.7 gramme atebrin weekly) be continued for a period of 23 days after the last bite where exposure to infective bites did not exceed 7 days.

The results of suppressive treatment in this experiment confirm these findings. It will be seen however from Table VI that B.T. parasites with overt malaria have been demonstrated some 20 to 50 days after cessation of

suppressive drugs in all the volunteers taking atebrin 0 1 gramme daily with or without sulphamerazine. Of the sixteen men taking atebrin 0 2 gramme daily fifteen have so far developed overt malaria. M T parasites have not been found in a single instance. Since suppressive treatment in these volunteers was continued for 4 weeks after the last infective bite it is a reasonable assumption that the *P falciparum* infections have been cured

The mean plasma atebrin level at the time of the overt attack for all the volunteers was 2·9 µg per litre the averages for the group varying from 2 2 to 3 4 µg per litre. Variations in concentrations in individual volunteers were 0 to 13 µg per litre

V VALUE OF ATEBRIN IN LOWER DOSAGE (0 3 AND 0 4 GRAMME WEEKLY) IN PREVENTING AND SUPPRESSING EXPERIMENTALLY INDUCED MALARIA IN MAN

Field experience had indicated that atebrin in a dosage of 0 3 and 0 4 gramme weekly not infrequently failed to suppress malaria In order to investigate the efficacy of atebrin in this dosage the following experiment was planned.

Three groups of volunteers taking atebrin in variable amounts were bitten by mosquitoes harbouring sporozoites of *P falciparum* or *P vivax*

The biting period has extended from one session with ten M T infective bites on 1 day to seventeen to twenty one sessions with B T and M T infective bites (Groups B and C) Detail of infection is set out in Table XI

Observations were made (1) during the period of biting (2) for 4 weeks after the last infective bite while still taking suppressive atebrin (3) for 7 weeks after ceasing atebrin. Subsequently subinoculation and susceptibility tests were performed where necessary

TABLE XI

INFECTIVE BITES FOR VOLUNTEERS ON SUPPRESSIVE REGIMENS OF ATEBRIN 0 3 0 4 AND 0 7 GRAMME PER WEEK.

Group	Number in Group	Number of biting sessions.	Duration of biting days	Infective Bites		
				Total *P falciparum*	Total *P vivax*	Total infective bites
A	6	1	1	10	—	10
B	7	21	85	15*–156	10*–108	259–264
C	8	17	69	144–154	79–80	223–234

In the combined series seven volunteers received 0·3 gramme, seven received 0·4 gramme and seven received 0·7 gramme atebrin weekly. The volunteers on the 0·3 gramme per week regimens received 0·1 gramme atebrin on Tuesday, Thursday and Saturday of each week, and in the case of the 0·4 gramme per week regimen an additional 0·1 gramme was given on Monday.

Groups B and C were subjected to the same stress and strain as already mentioned in Section III of this report, i.e. adrenalin and insulin injection, chilling and heavy exercise. Table XII sets out the results of the observations on these groups.

TABLE XII

THE VALUE OF SUPPRESSIVE ATEBRIN IN LOW DOSAGE IN TWENTY-ONE VOLUNTEERS
EXPOSED TO INFECTIVE BITES—*P. falciparum* AND *P. vivax*.

Group	Infection	Dosage of Atebrin in gramme per week	Number of Volunteers	Suppressive effect.			Curative effect after ceasing suppression.			
				Complete suppression.	Parasites in blood only.	Overt malaria.	*P. falciparum.*		*P. vivax.*	
							Presumptive cure.	Overt malaria.	Presumptive cure.	Overt malaria.
A	*P. falciparum*	0·3	2	1	0	1	1	0	—	—
		0·4		1	0	1	1	0	—	—
		0·7		3	0	0	0	0	—	—
B	*P. falciparum* and *P. vivax*	0·3	3	0	1	1	0	1	0	2
		0·4		2	1	0	1	0	0	2
		0·7		3	0	0	0	0	0	3
C	*P. falciparum* and *P. vivax*	0·3	3	0	1	2	0	1	0	2
		0·4	3	1	2	0	1	0	3	
		0·7	3	0	0	0	0	0	3	

In the seven volunteers taking atebrin 0·3 gramme per week, the build up in Group A was 43 days (two cases), in Group B, 15 days (two cases) and in Group C 40 days (three cases). The average plasma atebrin level was 8·8 μg per litre (arithmetic mean) and 6·9 μg per litre (geometric mean). The average minimum and maximum readings were 1·2 and 16·0 μg per litre.

In the seven volunteers taking 0 4 gramme per week, the build up' in Group A was 43 days (two cases) in Group B 15 days (two cases) and Group C 40 days (three cases) The average plasma atebrin level was 10 3 (arithmetic mean) and 9 1 μg per litre (geometric mean) The average minimum and maximum readings were 3 1 and 19 4 μg per litre

In the seven volunteers taking 0 7 gramme weekly the build up was 0·4 gramme for 3 days in two 0 7 gramme weekly for 28 days in five. The average atebrin plasma concentration was 24·0 μg per litre (arithmetic mean) and 21 4 μg per litre (geometric mean) The average minimum and maximum concentrations were 13 3 and 31 4 μg per litre

It will be seen that malaria has been adequately suppressed in

One of seven volunteers taking atebrin 0 3 gramme per week.

Four of seven „ „ „ 0 4 „ „

Seven of seven „ „ „ 0 7 „ „

P falciparum trophozoites appeared in the blood in six out of seven volunteers taking 0 3 gramme per week and in these gametocytes subsequently developed in four instances *P falciparum* trophozoites were demonstrated in three out of seven volunteers taking 0 4 gramme per week and in all three instances gametocytes later appeared. *A punctulatus* var *typicus* fed on one of these men were subsequently found to be heavily infected with sporozoites of *P falciparum* and were used to infect volunteers in other experimental groups.

In the seven volunteers taking 0 7 gramme atebrin per week neither asexual nor sexual parasites were ever found. The failure of 0 3 gramme and 0·4 gramme atebrin per week to cure the M.T infection in the volunteers who showed parasites in their peripheral blood, though they were quite fit enough to continue under the conditions of the experiment, is worthy of note

Throughout these experiments no volunteer taking 0·6 or 0 7 gramme atebrin per week has ever developed overt M T malaria if his suppressive regimen was continued for 23 to 28 days after the last infection In two volunteers 0 3 to 0 4 gramme atebrin per week though continued for 28 days after the last infection failed to cure the infection. The development of overt B T malaria in all the other volunteers with mixed infection is considered to be evidence of presumptive cure of the M T infection.

Certain features of interest may be cited —

(1) The failure of atebrin in a dosage of 0 3 gramme per week.

(2) The partial success of atebrin 0 4 gramme per week.

(3) The production of gametocytes in the partially suppressed volunteers of both these groups

(4) The fact that parasites of *P falciparum* have been exclusively found in all overt attacks while on suppression. It is remarkable that despite repeated infective bites from *P vivax* infected mosquitoes (107 in Group B and 80 in Group C) benign tertian parasites have never once

been demonstrated though exhaustive search of thick blood films has been made

Of Group A (ten infective M T bites) two on 0·7 gramme per wed one on 0·4 gramme per week and one on 0·3 gramme per week were completely suppressed while on atebrin. Some seven weeks later subinoculations with 200 c.c. of their blood into four other volunteers proved negative. Subsequently the intramuscular injection of blood containing M T parasites produced malaria in the four original volunteers, proving that none were naturally immune to *P falciparum* and none had acquired an effective immunity for the strain of M T parasites inoculated.

Comment

It is evident in the light of these experimental findings how reduction of standard suppressive atebrin to three or four tablets weekly may result in attacks of overt M T malaria, suppression of B T malaria, and the development of carriers in a force exposed to repeated infection in hyperendemic areas. In jungle fighting in the past, troops have generally taken atebrin but have done so irregularly and in inadequate dosage. This explains the disastrous epidemics of malignant tertian malaria in campaigns in New Guinea and elsewhere when they have been supposed to be on an adequate atebrin regime.

VI VALUE OF QUININE IN PREVENTING OR SUPPRESSING EXPERIMENTALLY INDUCED MALARIA IN MAN

Experiments similar to those used while investigating atebrin and the sulphonamide drugs were undertaken with volunteers taking quinine whilst B.P grains v and grains x per diem. The drug was administered daily as a mixture of quinine sulphate in ac. sulphone dil 1 ounce of this mixture containing grains x quinine. The appropriate dosage was given for 2 days prior to the first infective bite and continued daily thereafter The amount of quinine base in the mixture was checked by the photofluorometric method of BRODIE and UDENFRIEND Three groups have been investigated.

(A) MALIGNANT TERTIAN MALARIA.

Nine volunteers were exposed to 10 + infective bites at one biting session six volunteers had quinine suppression (three on grains x, three on grains v) two had atebrin 0·1 gramme per day and one (the control) received no antimalarial drug

The only result of giving quinine to these six men appeared to be some slight delay in onset of the attack of malaria. In the control, M T parasites appeared on the 9th, the fever on the 11th day In the quinine groups parasites were demonstrated on the 9th to 11th day in all cases and fever from the 11th to 16th day The spleen became palpable in all instances. One man biting

grains x quinine daily developed a moderate gametocyte wave (maximum 240 per c.mm.) In contrast to this the two volunteers taking atebrin (0 1 gramme daily) failed to develop either parasites or fever during the period of drug administration observations on them have already covered a period of 80 days, but are not yet completed as they are being subjected to repeated further infections

(B) BENIGN TERTIAN MALARIA.

The group used was similar to that used in Group A. They were exposed to 10 + infective bites at one session

In the control B T parasites appeared on the 12th and fever on the 13th day In the three volunteers taking quinine grains v daily parasites appeared on the 9th to 12th day and fever on the 12th to 15th day In the three volunteers taking grains x daily, one developed fever on the 12th day and demonstrable parasites on the 13th day Of the other two one developed headache and toxic pains and a temperature of 99 4° F on the 10th to 12th day unassociated with parasites in blood smears, and the other minor toxic symptoms only Up to the 30th day no other clinical or parasitological evidence of malaria was forthcoming and they then each received twenty infective bites (P vivax) In both instances parasites were observed nine days later (i e 39th day) In one, parasites persisted for 3 days only with a maximum density of 4 per c.mm. and in the other parasites persisted till the volunteer had overt B T malaria on the 54th day (24 days after his second infective bites) Both of these volunteers received six infective bites (P falciparum) on the 44th day and developed overt M.T malaria on the 63rd and 75th days (19 and 31 days after the M T bites) The two volunteers taking atebrin had developed neither fever nor parasites, and, despite reinfection on the 30th and subsequent days remained normal in all respects. They have been included in Section IV of this report (Series II XVIA)

(C) MIXED *P vivax* AND *P falciparum* INFECTION

A group of eight volunteers was used, two taking quinine grains v a day two grams x a day two on atebrin 0 1 gramme a day and two on atebrin 0 2 gramme a day They were exposed to 4 + infective bites B T on the first evening 4 + infective bites M T on the 6th and 12th evenings.

The results correspond with those in groups A and B—lack of suppression with quinine and complete suppression with atebrin.

In the B T control *P vivax* appeared in the blood on the 11th day and fever occurred on the 13th day following exposure. In the M T control *P falciparum* appeared in the blood on the 8th day and fever occurred on the 12th day following exposure.

In the four men receiving quinine (grains v and grains x) *P vivax* appeared in the blood and fever developed on the 13th to 14th day after first exposure

and *P falciparum* on the 14th to 16th day following first exposure, *i.e.* 18 to 22nd day of the experiment. It was noted that the parasitaemia was at first *P vivax* and later *P falciparum*—three of the four cases showing two distinct waves of pyrexia corresponding to the two waves of parasites. These men all developed considerable splenomegaly, hepatomegaly and anaemia, and quinine consistently failed as an anti malaria suppressant. In contrast to this, all four volunteers receiving atebrin (0 1 and 0 2 gramme) failed to develop demonstrable parasites or fever and remained perfectly fit throughout the period of observation which now equals 101 days.

Plasma Quinine Levels

Throughout this experiment estimations of the concentration of quinine in the plasma were made by the photofluorimetric method of BRODIE and UDENFRIEND. For this purpose blood was collected before the daily dose was administered and 2½ hours thereafter. The latter specimens gave values approximating to the maximum values attained with a dosage of v to x grammes daily. The results are epitomised in Table XIII

TABLE XIII

PLASMA QUININE LEVELS IN VOLUNTEERS
(DAILY DOSAGE GRAINS V AND GRAINS X).

	Grains V per day		Grains X per day	
	Minimum (before dose).	Maximum (½ hours after dose).	Minimum (before dose).	Maximum (½ hours after dose).
Number of men	15	16	11	11
Number of readings	64	23	63	30
Arithmetic Mean Quinine level mg per litre	0 78	3 77	0 77	4 5
Absolute Minimum Quinine level mg per litre.	0 18	1 4	0 2	1 0
Absolute Maximum Quinine level mg per litre	4	7 0	2 3	7 4

It will be noted that the average minimum and maximum values for the group taking grains v were 0·78 and 3 77 mg. per litre, and for the group taking grains x 0·77 and 4 5 mg per litre respectively

Comment

It is evident from these observations that quinine given in doses of 5 or 10 grains a day is inadequate to suppress the strains of *P vivax* and *P falciparum* found in Papua when inoculated by mosquitoes into healthy volunteers.

The contrast between atebrin and quinine is striking proving as it undoubtedly does the vast superiority of a daily dose of 0 1 gramme atebrin over grains x of quinine in both *P vivax* and *P falciparum* infections

In the Milne Bay and Buna Gona campaigns epidemic malignant tertian malaria predominated up to January 1942, when quinine began to be replaced by atebrin. Viewed in retrospect failure of suppressive treatment now appears to have been inevitable, since even if taken in the advocated dosage of grains x daily this drug is incapable of preventing break through in M T infections

The intensity of infection as estimated by the number of infective bites is higher than would be anticipated in the jungle even in hyperendemic areas, and the failure of quinine might be related to this fact as well as to the virulence of the Papuan strain or strains of *P vivax* and *P falciparum* used in these experiments It is proposed at a future date to investigate the suppressant action of quinine in milder experimentally induced infections *i.e* one to three infective bites

VII MALARIA IN NEW GUINEA (1944)

In the introductory section a brief reference was made to the appallingly high malaria casualties in Australian troops in the New Guinea campaigns of 1942-1943 Similar high malaria casualty rates were experienced by U S A troops in the Philippines at Bataan and in Guadalcanal and in British and Indian troops in Assam and Burma. In the Australian Army the very pertinent question was continually raised by field commanders and staff officers regarding the real efficacy of the anti malarial measures advocated by the Medical Directorate and whether suppressive or prophylactic drug treatment could in fact control repeated malaria infections in the stresses and strains of arduous jungle fighting The experiments as outlined in this paper were devised and carried out to get relevant data which would answer this question. These data have now been officially accepted as proving that a correct atebrin regimen will lead to the control of malaria in hyperendemic areas and enable troops to fight on in the jungle with an absolute minimum of malaria casualties. Knowledge regarding atebrin administration, personal protection and other anti malaria measures is now recognized as an essential part of a soldier s training for jungle warfare, and the institution of anti malaria measures and atebrin suppressive medication have become a matter of strict military discipline.

Following this there has been a dramatic fall in malaria casualties in troops in both base and forward line areas in New Guinea. Thus, in December 1943 the malaria rate was 740 per 1 000 per annum by November 1944 this had

fallen to 26 per 1 000 per annum as shown in the accompanying Graph. This remarkable achievement has not been solely due to atebrin, for less hard fighting was going on later in 1944 conditions were more static and in consequence conditions for destruction of adults and the control of larvae were much more favourable   Most of these troops however had at some time or another acquired *P vivax* infection, and the absence of frequent relapses can only be explained by a very high standard of atebrin discipline which has not been attained.

In addition to a preliminary build up the regimen included a continuation of suppressive treatment (0 1 gramme daily) for 4 weeks after leaving malarious
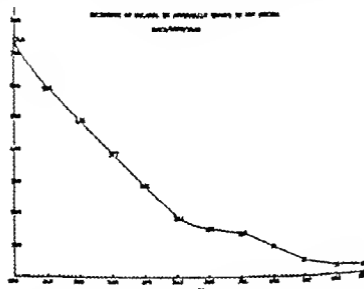
GRAPH 4 —Fall in hospital admission rate for malaria in New Guinea in Australian troops from December 1943 to November 1944.

areas. If this were done our experimental results indicated that there would be no fatal cases of malignant tertian malaria and no blackwater fever and cure of *P falciparum* infections would be attained.   This result has now practically been achieved in the field as blackwater fever or deaths from acute pernicious malaria are both extremely rare   For the past 2 years in the Australian Military Forces the death rate for uncomplicated malaria has not exceeded 1 in 3 000

VIII SUMMARY

In epidemic malaria in New Guinea most Australian troops contracted both *P falciparum* and *P vivax* infections. During active jungle fighting malignant tertian infection was the major cause of malaria casualties later after

treatment, relapses occurred with great frequency due to *P vivax* Apart from seasonal considerations twn factors have contributed to this (a) the fact that *P vivax* infections require a lower dosage of atebrin or quinine for suppression (b) when the two species are in competition on equal terms *P falciparum* breaks through and *P vivax* is suppressed. This can be shown experimentally in volunteers receiving the same number of infective bites (*P falciparum* and *P vivax*) on the same day under these circumstances primary malaria fever is due to *P falciparum* thus being the only parasite demonstrable in blood smears after a course of treatment, relapses occur but these are due only to *P vivax*

The strain or strains of *P vivax* encountered in New Guinea and New Britain differed from the strains encountered in Australian troops in the Middle East and those previously worked with experimentally in U K. and U.S.A. inasmuch as (1) the relapses appear within a few weeks of primary fever or the cessation of suppressive drug therapy (2) though febrile attacks are readily controlled by anti-malarial drugs the subsequent relapse rate is unduly high.

Subinoculation

Subinoculations by direct blood transfusion using a minimum of 200 c.c. of blood have been extensively used both in controls and test cases (*i.e.* volunteers exposed to infective bites while taking suppressive drugs) in order to determine the infectivity of whole blood at different stages in the infection.

Seven minutes after biting has ceased on one arm, direct transfusion of blood from the other arm has produced malaria in the recipient in both *P falciparum* and *P vivax* infections To date no definite positive subinoculations have been recorded in blood collected 30 minutes after biting has ceased. Evidently sporozoites after inoculation rapidly gain access to the circulating blood and are almost as rapidly removed presumably by macrophages and fixed tissue endothelial cells.

In *P falciparum* infections, blood (200 c.c.) collected during the first 6 days invariably failed to transmit malaria whereas subinoculations from the 7th day onward yielded positive results In *P vivax* infections subinoculations were invariably negative until the 9th day, when they also yielded positive results.

If the tissue cycle be 48 hours the sharp line of demarcation between the negative and the positive subinoculation results in M.T and B T respectively suggests that there are four cycles in *P vivax* and three in *P falciparum* before the metacryptozoites liberate erythrocytic parasites into the circulation

The persistence of the tissue stage (exoerythrocytic stage) in *P vivax* is indicated by the reappearance of the erythrocytic forms after demonstrable parasites have disappeared and subinoculations become negative Similarly

the tendency of B T infections to relapse repeatedly despite prolonged anti-
malarial treatment might be best explained in terms of such a mechanism.

In M T malaria the fact that the disease is readily cured and that even
negative subinoculations become established recrudescences do not occur,
indicates either that the tissue phase is of short duration or that atebrin acts on
the exoerythrocytic forms as well as the asexual blood parasites.

Data on subinoculations also suggest that when investigating an action of
drugs on (a) sporozoites the peak concentration should be at the time of
biting   (b) early tissue stage in cryptozoites and metacryptozoites the drug
should be administered for the first 6 days in P falciparum, and for the first
8 days in P vivax infection   (c) asexual parasites the drug should be adminis-
tered from the 7th day onwards in P falciparum and from the 9th day onwards
in P vivax infection. In drugs like atebrin which have a cumulative action the
interpretation of results may be difficult because the   die away is slow

Suppressant Drugs

In routine experiments on volunteers to infection the standard treat-
ment adopted to test chemotherapeutic suppression and prophylaxis in malaria
consisted of the administration of the drug for a variable period prior to
infection throughout the period of exposure to infection and for 23 days after
the last infective bite   The latter figure was regarded as covering the upper
limit of the incubation period of P falciparum and P vivax as far as primary
malaria fever was concerned   After cessation of daily suppressive treatment
volunteers were watched for another 35 to 40 days and if there was no clinical
or haematological evidence of malaria, subinoculations (200 c.c.) were made
in all P falciparum infections   If the recipient failed to get malaria and no
fever   or parasites had developed in the first volunteer (donor) during the
ensuing period he was considered free from malaria. In order to eliminate
the possibility of his being insusceptible or naturally immune he was then
given an intramuscular injection of blood containing an estimated number of
malaria parasites (P falciparum). If malaria ensued, as invariably it did, it
proved his susceptibility to malignant tertian malaria and also the absence of
any effective immunity to the strain of parasite inoculated.

When investigating drug action it became customary in a certain proportion
of volunteers to make early subinoculations (M T from the 7th and B T
from the 9th day   onwards) in order to determine whether asexual parasites
were appearing in the blood and, if not whether their appearance was merely
delayed, or they were permanently absent. By this procedure valuable data
were obtained regarding the phase of the parasite on which a given drug was
acting and information was often obtained as to whether infection had actually
occurred. The action of certain sulphonamide drugs including sulphadiazine,
sulphamerazine and sulphamezathine were very completely investigated. With

standard suppressive regimen in doses of 1·0 gramme daily these drugs were found to suppress twenty and cure seventeen out of twenty one mosquito-transmitted infections, in *P falciparum* infections. Similar results were obtained in blood inoculated malaria (trophozoites) and from these and other findings it was evident that these drugs act very effectively on the erythrocytic asexual parasites. On the other hand they failed in *P vivax* infections, for twenty-one out of 24 volunteers developed overt malaria while taking the drug and the remainder broke down with malaria shortly after drug administration ceased.

The action of atebrin alone or in combination with sulphamerazine was investigated in a detailed manner against (1) *P falciparum*, (2) *P vivax* (3) mixed infection with both species of parasite. In the dosage adopted the combination of atebrin and sulphamerazine showed no advantage over atebrin alone.

In volunteers infected with *P falciparum* on a standard atebrin regimen (0·6 to 0·7 gramme atebrin weekly) malaria suppression was adequate, and provided the drug was continued for the prescribed period cure invariably resulted. In *P vivax* infections a similar atebrin regimen was effective in preventing overt attacks of malaria but within a few weeks of cessation of atebrin suppression an attack of benign tertian malaria developed.

When volunteers on this atebrin regimen (0·7 gramme weekly) were repeatedly infected with *P falciparum* and *P vivax* overt attacks of malaria still failed to develop provided the daily dose of 0·1 gramme was regularly taken. Later after suppressive treatment ceased they broke down with B T but never with M T malaria Then volunteers were exposed to many more infective bites over a period approximating to 3 months than they would be ever likely to encounter in a hyperendemic area of malaria in the jungle In addition many were subjected to hard physical work and prolonged marching up and down hills in a tropical climate to extreme cold at 0° F for I hour and anoxia similar to that experienced when flying at altitudes of 15 000 to 19 000 feet without oxygen. Insulin was given to lower the blood sugar simulating the hypoglycaemia of semi-starvation and big doses of adrenalin were injected to reproduce the hyperadrenalism caused by emotional states characterized by fear and anger Overt attacks failed to develop despite everything that was done to precipitate malaria breakdown. The military implications of these experiments were considerable for they showed that non immune troops on a correct atebrin regimen could be brought into hyperendemic areas of malaria and be engaged in the fighting in the jungle for many months with an absolute minimum of malaria casualties. Since gametocytes never appeared in the blood there would be no malaria carriers in the force, and as malignant tertian malaria was cured there should be no deaths and no blackwater fever the only problem which remained was that of latent benign tertian malaria which would produce overt attacks a few weeks after atebrin suppressive treatment ceased. Subsequent field experience in New Guinea and other theatres of war has fully

confirmed these conclusions reached in these large scale experiments on human volunteers (*vide* Graph 4 page 350)

Similar experiments were undertaken in volunteers taking quinine sulphate (B P) 5 grains and 10 grains daily In *P falciparum* infections overt malaria developed in every volunteer taking 5 or 10 grains of quinine daily In *P vivax* infections 5 grains of quinine failed entirely whereas 10 grains proved more effective but sometimes failed. In mixed infections the volunteers taking quinine all developed splenomegaly hepatomegaly anaemia and blood parasites, and quinine had to be regarded as failing as an anti-malaria suppressant—at least as far as the New Guinea strains of *P falciparum* and *P vivax* were concerned.

IX. CONCLUSIONS.

The conclusions reached regarding chemotherapeutic suppression and prophylaxis in volunteers infected with New Guinea strains of malaria and following the drug regimen advocated in this paper are as follows :—

(1) Quinine sulphate (B.P) even in a dosage of 10 grains daily failed to prevent overt attacks of M T malaria. Quinine sulphate in a dosage of 5 grains daily is incapable of preventing attacks of overt benign tertian malaria, but when the dosage is increased to 10 grains daily complete suppression is afforded in some cases but not in others.

(2) Certain sulphonamides (sulphadiazine, sulphamerazine and sulphamezathine) in a dosage of 1·0 gramme daily suppressed malaria in twenty out of twenty-one volunteers infected with *P falciparum* and cured seventeen out of twenty-one. They act not as causal prophylactics in man, but by destroying the asexual blood parasites.

(3) The same group of sulphonamides in the same dosage failed as a suppressant in *P vivax* infections, overt malaria appearing in twenty-one out of twenty four volunteers during the period of drug administration. The remaining three volunteers developed benign tertian malaria shortly after the administration of the drug ceased.

(4) Atebrin in a dosage of 0 1 gramme daily suppresses malignant tertian fever and if continued for the requisite period after the last exposure to infection, cures the disease. Subinoculation tests prove the action to be on the asexual blood parasites.

(5) Under similar circumstances atebrin suppresses benign tertian malaria, but overt malaria supervenes with great regularity a few weeks after suppressant atebrin administration ceases.

(6) In mixed hyperinfections (*P falciparum* and *P vivax*) suppression is equally effective under a similar atebrin regimen it is always B T and never M.T malaria which breaks through when atebrin suppressive treatment ceases.

(7) Various factors such as hard physical work, marching up and down hills, swimming extreme cold, anoxia, blood loss, and injections of adrenalin

and insulin have completely failed to produce malaria breakdowns in infected volunteers.

(8) The military implications of these experiments are discussed. It is shown that if the conclusions drawn be correct and granted infallible atebrin discipline, it should be possible to fight a non immune force for many months in hyperendemic areas of malaria without significant malaria casualties There should be no malaria carriers the death rate should be 0·0 per cent. and blackwater fever should not develop The residual problem would be one of relapsing B T malaria.

(9) Field experiences in New Guinea during 1944 are cited as generally confirming these conclusions the hospital admission rate there having fallen from 740 per 1,000 per annum in December 1943 to 26 per 1 000 per annum 10 November 1944

DISCUSSION

Colonel S P James (in opening the discussion) I am very sensible of the honour of being invited to open the discussion on Brigadier FAIRLEY's highly important paper As we all know a great obstacle to malaria research has always been that no laboratory animal is susceptible to the human disease That obstacle was partly overcome when malariatherapy was introduced, for the practice justified the same kind of experimental research on malaria in the human subject as would be conducted if a suitable laboratory animal were available. But the patients for whom malaria therapy is prescribed are not normal healthy individuals and the sheltered conditions in which they live in hospital in England are very different from those of residents in the tropics. So it has often been said that the results of anti malarial therapeutic trials on general paralytics in England may not be applicable to the disease in young healthy people living and working in malarious regions Of course when those experiments on mental patients were begun a good many years ago, no one dreamed that a time would come when they would be repeated on healthy soldiers at war in a very malarious part of the world. Brigadier FAIRLEY is to be congratulated sincerely on his successful accomplishment of this task and on having obtained results which permit no doubt of the efficacy and practicability in the field of the protective measures tested. I think we should congratulate him, too on his success in obtaining so many volunteers for the experimental trials, and that a tribute is also due to the volunteers themselves for their self-sacrificing co-operation. It seems to me that this new practice of using healthy volunteers instead of hospitalized mental patients marks quite an epoch in the history of malaria research. A few isolated examples are to be found in the malaria literature of many years ago as, for instance, in 1900 when Sir PATRICK MANSON's son and a technical assistant were voluntarily given malaria by the bites of infected mosquitoes in order to provide a practical

demonstration of the truth of the   Mosquito-Malaria Theory   But, on a sufficient scale for therapeutic trials, I think the practice may be said to have begun last year when young men belonging to the Friends Ambulance Unit under Major KENNETH MELLANBY volunteered to submit to malignant malarial infection for the trial of prophylactic drugs by the Horton group of workers. That was a patriotic and unselfish act which merits high praise.

Brigadier FAIRLEY's results, apart from their immediate practical value to the war effort, are of great interest from the laboratory worker's point of view   I should like to remark briefly on one or two of them.   First there is his finding that rises of temperature which are called spikes or peaks, occur at intervals in the volunteers on the prophylactic regime of 0·1 gramme of mepacrine daily   No malaria parasites were found in the peripheral blood during these transient febrile attacks.   In our original work at Horton we recorded similar findings in persons on a prophylactic regime of 5 grains of quinine daily and the present Horton group of workers had the same experience in their experimental trials of mepacrine last year   Does Brigadier FAIRLEY consider these spikes to be minor attacks of malaria?   I do not think that, in persons on a prophylactic regimen of mepacrine, this question can be answered by subinoculation, because in routine malaria therapy practice and in laboratory work on avian malaria, it is a common experience to have failures to infect even when the donor's blood contains many parasites and no mepacrine. Perhaps a surer way to get a correct answer would be to stop the prophylactic doses at the first rise of temperature and to continue to take temperatures and blood films daily for some time.   A second interesting finding was that a clinical B T attack always occurred about 90 days after stopping the mepacrine course.   A precisely similar experiment with the Madagascar or other strain of benign tertian does not seem to have been made previously so the significance of the finding remains doubtful, but I have the impression that the latent period of B T malaria contracted in the Mediterranean theatre and suppressed by mepacrine is usually much longer than 30 days.   A comparative series of prophylactic and therapeutic tests with the New Guinea and Madagascar strains is therefore very desirable and should yield important results. In the same connection Brigadier FAIRLEY's finding that the New Guinea strain of B.T is not suppressed by a prophylactic regime of 10 grains of quinine daily is also most important   Indeed, his results in general support the view expressed at a meeting of our Society about 10 years ago that, from the point of view of drug prophylaxis and treatment, strains may be more important than species

Lastly I must mention Brigadier FAIRLEY's experiments to ascertain on what phase of the parasite mepacrine acts.   They are of much interest, especially the finding that in persons who take a daily dose of mepacrine, parasites can be demonstrated in the peripheral blood on the 9th day after infection but that they disappear by the 12th day   It would be interesting to know whether the parasites which can be found on the 9th day are pigmented or not.

Evidently mepacrine, like quinine, cannot prevent a few parasites from appearing in the peripheral blood during or at the end of the incubation period. They are the parasites which KORTEWEG SWELLENGREBEL and others found were not affected by quinine And they are the parasites which led WARRINGTON YORKE to conclude that quinine does not begin to act until a good many parasites are present in the peripheral blood and which led SWELLEN-GREBEL to conclude that quinine has no action in a primary attack of B T malaria until the end of the period of initial fever defined by Dutch clinicians Are they the same kind of unpigmented parasites as those found by Miss BISHOP in canaries at the end of the incubation period of sporozoite infections with *P relictum* which were being suppressed with maximum tolerated doses of atebrin? If so it seems to me that Brigadier FAIRLEY's finding affords further support to the view that in B T malaria the first parasites which appear in the peripheral blood are unpigmented merozoites and trophozoites of an exoerythrocytic primary tissue-phase cycle which is not susceptible to any known anti malarial drug The disappearance of parasites by the 12th day would be explained by saying that in the interval between the 9th and 12th days the parasites of the primary tissue phase cycle would have sporulated once or twice so that the red cells would now contain only the ordinary type of erythrocytic parasite which is very susceptible to mepacrine Brigadier FAIRLEY's results show once again that as yet we do not possess a drug that will prevent *infection* although we have several which like mepacrine, will suppress the clinical and, to some extent, the parasitological effects of that infection. In other words, no true causal prophylactic is yet available.

Mr P G SHUTE   Colonel JAMES has paid tribute to the tough Australians who volunteered to become experimental subjects to be infected with, and in many cases suffer from, malaria. He mentioned too that in England members of the Friends Ambulance Unit also volunteered.

I should like to put on record a similar tribute to the British Tommy Last year I flew to Italy, taking with me 1 000 English anopheles mosquitoes for the purpose of infecting them with malignant tertian malaria and bringing them home alive for experimental work. On arrival at a military hospital in Naples, I explained to the Commanding Officer that I wanted suitable volunteers who would be willing to be bitten daily for several days by hundreds of mosquitoes. The C.O took me to one of the wards containing fifty to sixty patients and after explaining the purpose of my mission volunteers were called for every one a bed patient and suffering from malarial fever Nearly all of the patients volunteered and I had no difficulty in infecting a thousand mosquitoes and bringing them home where, by infecting further British Tommies who had volunteered we were able to obtain valuable information about the Italian type of malignant tertian malaria.

Lieut.-Colonel E H Vere Hodge   Brigadier Farley has explained why it is that, in this country we see amongst men returning from the tropics relapses almost exclusively of benign tertian malaria. Now the question arises is it worth while continuing prolonged treatment in these cases or having controlled the fever should we stop there? Various courses of quinine, mepacrine and pamaquin have been advised and employed Lately a 21-day course of quinine and pamaquin has been suggested but results seem to show that none of these courses seriously prevents relapses.

Dr George Macdonald   I would like to thank Brigadier Farley for his address on a piece of work which will materially help the war in all tropical areas. Since Colonel James raised the question of strains of parasites a few minor pieces of evidence from the Mediterranean might be relative. First we have definite evidence that atebrin, if actually consumed in the doses stated, will suppress benign tertian malaria in exactly the same way with the Mediterranean strain as with the New Guinea strain. I have two controlled pieces of evidence. The first one is in Syria on troops, where from an adequate control we had sound reason to believe they should have 100 per cent. of malaria cases per month. The administration of atebrin was carefully controlled and the actual incidence over a period of 4 months came to under 1 per cent. per month. Secondly in Italy I am sure that Professor Missiroli would permit me to mention that in the Maccarese area he has a population of about 5 000 people receiving atebrin which is actually administered by State nurses. Naturally some of the people have refused and some have turned out to be irregular patients. Owing to destruction of control work by the Germans the transmission is high. Amongst the regular takers throughout the period from June to September the incidence amounted to about 0·1 per cent. amongst irregular takers to 8 per cent. and amongst non-takers to 16 per cent. On the question of the period after cessation at which relapses occur, it has been the previous experience in Mediterranean areas where no suppressive treatment is given that there is a summer rise during the period from July to October followed by a decrease to negligible figures until the following transmission season in the following June Those parts of the Mediterranean where we have relied to a certain extent on suppression as a means of control have a certain number of cases during the transmission period. The mepacrine has been discontinued at the beginning of November after transmission has stopped the cases are declining about this time, and they remain at negligible figures during the period November to February Towards the end of February they increase, and there is a definite epidemic during the months of February March and April. This spring rise, at a time when there is no transmission, is sometimes high, and I attribute it entirely to the occurrence of relapses at an interval of several months after stopping suppressive treatment, as described by Colonel James, but whether the difference from the South-West Pacific

experience is due to different strains of parasite or to a difference of climate I do not know

Brigadier FAIRLEY shows conclusively that troops in highly malarious areas must be given atebrin, and that it is a most important means of controlling malaria but I feel strongly that in rear areas the administration of atebrin is a mistake. Malaria can be controlled by other means, and if atebrin is given the consequent suppression masks the fact that infection is going on. Infections are received, they are masked at the time but the patient subsequently goes down, and we have had the position of troops on operational work going down with malaria which they had acquired in the base area, when it was not recognized that they were infected. I believe this has occurred amongst troops brought home from the Mediterranean and sent to other theatres of war If atebrin had not been given there would have been slight epidemics, these would have been recognized, adequate measures would have been taken, and the ultimate number of cases would thereby have been reduced.

Major James Reid Brigadier HAMILTON FAIRLEY's work has been followed with the greatest interest in this country We have been studying the same problems with reference to M.T malaria but in a different way because in our initial investigations most of our volunteers could be under observation for limited periods. Accordingly we have tried to find the blood level of mepacrine that suppressed malignant tertian malaria in 50 infected volunteers on a variety of mepacrine regimes. Our estimations of mepacrine were made on whole blood instead of plasma, which Brigadier FAIRLEY and most other workers have chosen for their estimations In whole blood we found that if the mepacrine level fell below 80 μg per litre, M.T malaria always broke through above this level the disease was effectively suppressed. We next asked ourselves if a dose of 0 1 gramme daily would keep the blood mepacrine above this critical level of 80 μg per litre for an indefinite period. To test this, we have made blood mepacrine estimations at frequent intervals on the twelve volunteers who were observed to take a daily 0 1 gramme tablet of mepacrine for from 2 to 7½ months. In these men we found, in agreement with Brigadier FAIRLEY s observations on plasma mepacrine that there is a peak concentration between 1 and 2 months from the start of dosage The plasma concentrations reported by Brigadier FAIRLEY have remained constant at this peak level, but our whole blood concentrations have shown a progressive fall from the peak level to one of about 100 μg per litre at 6 months after dosage began. This is still above what we regard as the critical suppressive level of 80 μg per litre, but we consider that some of the single values recorded were coming too near the critical level to leave an adequate margin of safety It may be of value in field work to do urinary estimations, which are much simpler than blood or plasma estimations because we have found throughout our observations that the whole blood and urinary values ran in parallel curves.

Among our fifty infected volunteers, thirty nine were on mepacrine dosage regimes comparable to that used by Brigadier FAIRLEY our volunteers took 0 1 gramme daily for 6 days each week after a preliminary build up. Unfortunately we were not able to supervise mepacrine administration as he had to accept the word of our volunteers that they took the drug as instructed for they were in many different places. They were infected by mosquito bites in the same way as Brigadier FAIRLEY s men and we are able to confirm the suppressive action of mepacrine on malaria. One volunteer went down with malaria and his blood mepacrine level was then only 70 μg. per litre. As I have explained, we regard 80 as a minimum suppressive level in whole blood. In one other volunteer who had pyrexia for 2 days only a single parasite was found in a thick film. Pyrexia without detectable parasites was recorded in many of our volunteers but the interpretation of this finding was difficult because influenza was prevalent at the time and other patients in the same wards as our volunteers were also having pyrexial colds and influenza without having been infected with malaria. On sorting out the pyrexias among the thirty nine comparable volunteers we felt that six could be regarded as possibly malaria manifestations these pyrexias were similar in every way to the splenic of temperature that Brigadier FAIRLEY recorded in a few of his infected volunteers on mepacrine. These pyrexias produced only minor incapacity and our purpose in drawing attention to them is to emphasize that, in the field, pyrexias of unknown origin and short duration must be differentiated from frank malaria, otherwise men will be needlessly evacuated when in reality they are fit to return to duty after 24 to 48 hours. Mepacrine is clearly an essential drug to a force in a malarious area it has reduced the death-rate from malaria and allowed troops to operate where without mepacrine this would have been impossible.

Finally it may be emphasized that the results of the experimental investigations undertaken in this country on malaria-infected volunteers taking suppressive mepacrine in a dosage of six or seven tablets per week, are in complete agreement with those obtained by the Medical Research Unit, L.H.Q. Australia. The majority of our volunteers were infected with Roumanian malignant tertian malaria and there is no valid evidence that this strain used in England is mepacrine resistant or behaves differently from the New Guinea strains of M.T. malaria so far as mepacrine suppression is concerned. It is true that one of our volunteers developed clinical malaria and parasites when the blood level was reduced to 70 μg per litre. Unfortunately in these experiments mepacrine was not given under medical supervision, and, as in the case of the two volunteers in Brigadier FAIRLEY s series, the possibility cannot be excluded that the presence of numerous parasites associated with low mepacrine values may have been due to failure to take mepacrine.

Brigadier G M FINDLAY   Perhaps a few facts from West Africa may be of interest to compare with those that Brigadier FAIRLEY has reported from Australia.

In West Africa, still a hyperendemic malaria area, it is of course impossible to carry out experimental infections in human volunteers since there is considerable risk of becoming infected either in Freetown on arrival or shortly afterwards. Despite drainage schemes there are still many infected mosquitoes as is shown by the fate of a large number of Lascars who were landed at a West African port and were not given mepacrine as a suppressive within 6 weeks of arrival nearly two-thirds of them were in hospital with malaria.

A number of field experiments on suppression with mepacrine and with the sulphonamides have been carried out. In May 1942, units were divided into two, one-half continuing with 5 grains of quinine daily the other taking 0·4 gramme of mepacrine weekly Those on mepacrine had approximately 400 attacks of malaria per 1,000 strength those on quinine 600 attacks. When the dosage of mepacrine was increased to 0·6 gramme daily the results were more satisfactory Even more striking than the reduction in the incidence of malarial attacks is the almost complete disappearance of blackwater fever when mepacrine is used as a suppressive and mepacrine only is used for treatment of malarial attacks. In the past year there have been only two cases of blackwater fever in Europeans one was in an officer who, contrary to orders took quinine as a suppressive and quinine for four malarial attacks. The conditions necessary for the occurrence of blackwater fever have not disappeared for among African troops an increase in blackwater fever has taken place.

The question of the mepacrine concentration of the plasma after some months on mepacrine suppression has been raised. So far as our results go there is no evidence of any diminution in mepacrine concentration of the plasma In fact, if liability to a clinical attack is closely correlated with the mepacrine concentration of the plasma, it would seem that it is in the early months of mepacrine suppression that there is most likelihood of a breakdown. An analysis of 850 officers and men who were presumably taking 0·6 gramme mepacrine weekly showed that over 60 per cent. had their first clinical attack within 3 months of beginning mepacrine suppression if 8 months were passed without any attack it was very rare for a primary attack of malaria to occur during the next 10 months I quite agree with Brigadier FAIRLEY in saying that the vast majority of breakdowns are due to failure to take mepacrine but there are a number of cases where failure to take mepacrine can be excluded. A senior medical officer on his way back to West Africa from leave in South Africa had a severe attack of malaria although he had continued most carefully to take 0·1 gramme of mepacrine daily

Both therapeutic and suppressive tests have been carried out in West Africa with sulphonamides. Many sulphonamide compounds have some therapeutic action in malignant tertian malaria, although they are not as active as mepacrine. Small scale experiments with sulphamezathine, sulphamerazine and one other compound have been instituted, the daily dose being 0 5 gramme. Sulphamezathine was about equal to mepacrine, sulphamerazine rather less

efficient than mepacrine   0 5 gramme tablets have been taken daily for over a year without any evidence of toxic action.

Professor B Maegraith   Mr. PRESIDENT since the services of volunteers have been mentioned by previous speakers, I should like to add my tribute to the undergraduate volunteers I have had working with me in Oxford. In the course of the last 18 months some 450 undergraduate men and women have assisted to the experiments of the Malaria Research Unit. These volunteers have not been infected with malaria but they have had to submit from time to time to considerable discomfort and hardship and I would like to say that I have had the utmost co-operation from them. As a result of their help we have been able to gain much valuable information about the pharmacology and toxicity of mepacrine, information which has been of some practical importance

Our findings with regard to mepacrine levels in the blood and plasma do not coincide with those of Major REID   It is not our experience that plasma or whole blood mepacrine levels fall off after up to 15 months on suppressive doses (0·1 gramme daily) of the drug nor have we observed any increased output in the urine over similar periods. I think our results are similar to those of Brigadier FAIRLEY and the American workers.

Finally may I congratulate Brigadier FAIRLEY on providing such an excellent example of the immense practical value of scientific research.

Brigadier J A Sinton congratulated Brigadier FAIRLEY and his team upon the conclusive results obtained by experiments so clearly planned and brilliantly executed. He also desired to thank him on behalf of the Army for the irrefutable evidence produced as to the outstanding value of mepacrine suppression during military operations in the tropics. While most of us were already convinced of this, the layman, and even many medical men, had put forward numerous suggestions to account for serious malarial "break throughs reported, while an adequate dosage of suppressive mepacrine was supposed to have been taken conscientiously The work in Australia has, we hope, now definitely refuted the suggestion that significant outbreaks of malaria can occur while mepacrine suppressive treatment is being properly taken. His results go to confirm the evidence collected from many other theatres of war that the main, and probably the only cause of failure of such treatment depends upon failure to take the drug regularly and unfailingly at the dosage ordered, i e the results depend upon the efficacy of anti-malarial discipline.

I would also like to add my appreciation to those of other speakers, who have praised the self-sacrifice of the many volunteers, both military and civilian, who have so willingly given their services in this country to enable trials of anti malarial drugs being carried out.

There is no doubt that, for use among non-immune populations mepacrine is the best suppressive drug which we know so far and is much superior to quinine in this respect. In view of the findings of Brigadier FAIRLEY with quinine, one wonders why such relatively good results with this suppressive have been reported in the past from all quarters of the world. If one considers the populations who formerly depended upon quinine suppression, one should realise that the majority of these persons had had attacks of malaria at some time, and, as a result, had developed a varying degree of immunity to the clinical effects of malarial infection. In such individuals, the pyrogenic threshold of parasite prevalence is markedly raised* and in consequence a less potent drug would be capable of keeping clinical manifestations suppressed in most circumstances.

Quinine has still many uses in malaria therapy. Colonel VERE HODGE has spoken about the poor results obtained in the radical treatment of chronic benign tertian malaria. Such cases form a serious problem to which the answer has not yet been found in all instances. These difficult cases have been the subject of large scale research in this country with different anti malarial drugs either alone or in combination. So far the results obtained by a 10-day course of the old quinine-plasmoquine treatment, used so successfully in India about 10 years ago have shown themselves three to four times as good as any other system of treatment advocated (including massive dosage with mepacrine). The infections were mainly with Mediterranean strains of *P. vivax* so it has still to be determined whether equally good effects can be produced against strains from other regions.

Sir Leonard Rogers With reference to the value of research volunteers one group of persons has not been thanked. Our greatest thanks are due to the German discoverers of atebrin who have enabled us to fight their friends the Japanese.

Brigadier Fairley (in reply) In regard to these spikes in temperature some of our volunteers in the absence of demonstrable parasites had a certain amount of clinical evidence that they were harbouring malaria parasites. Occasionally tender or palpable spleen or liver was noted, and occasionally they developed transient elevations of temperatures which were possibly due to the malaria parasite attempting to break through without success but they were never ill enough to go to bed and continued their routine duties. But malaria only accounted for a proportion of pyrexial reactions that occurred. Not infrequently during the control period *before they were exposed to malaria* it was noted that volunteers developed transient pyrexia (spikes " in the temperature chart) which at times were unexplainable. These men were in the tropics, doing heavy exercise, taking long marches in the heat of the day

*SINTON J A, et al (1931) *Ind J med Res* **18**, 871

or cutting wood 5 days a week, and they sometimes developed transient elevations of temperature as the result. For example, one particular "spike in the temperature chart was due to the fact that this man and another were getting a little tired of the experiment: they did not stop taking atebrin, but decided to do their best to break down with malaria. They went up into the hills: were away many hours, walked a very long distance and came back thoroughly fatigued and with sore, blistered feet. But no malaria developed and parasites were not demonstrated. Upper respiratory tract infections were another common cause of transient pyrexia, and sometimes localising features were so mild that they could be readily overlooked. Our criterion of "break down" was that a man must have a temperature of 100° F or over be rich enough to go to bed and have demonstrable malaria parasites in blood smears. The morale of these volunteers was very good and they never went to bed unless they felt really ill. Most incredible things can be done during a malaria attack (P. vivax). One of these men with a benign malaria attack and a temperature of 103° F got the top score in a cricket match. Subinoculation from volunteers who have been infected with P. falciparum and are taking 0.1 gramme of atebrin a day are positive on the 7th, 8th and 9th day and with P. vivax on the 9th, 10th and 11th day following exposure without necessarily showing any elevation of temperature. Parasites at these times are not demonstrable—even in thick blood smears. In our experience, which now is very considerable both in the field and in experimentally infected volunteers, men are not seriously incommoded with malaria in the absence of demonstrable parasites in thick blood films. When taking 0.1 gramme of atebrin daily clinical manifestations will be so mild and pyrexia, if it occurs, so transient, that they will not lead to confusion in the differential diagnosis of P U O in the forward areas. When more serious clinical features and fever develop due to malaria, the man will naturally be evacuated and parasites will be found in thick films if a sufficiently careful examination be made by a competent pathologist. One is here dealing with an attack of overt malaria, and, in our experience, this occurs only when the dosage of 0.1 gramme of atebrin daily has been inadequately and irregularly taken. Our volunteers were far more heavily infected with both P. falciparum and P. vivax than they ever would be in the jungle and they were subject to most arduous physical work, yet they never became hospital casualties.

The next point was raised by Dr MACDONALD. Chemotherapy and atebrin suppression are not yet so effective that we can afford to disregard other anti-malarial measures such as personal protection and the destruction of mosquito adults and larvae. That position would only arise if we had a cure for P. vivax infections. Actually in the South-West Pacific infected troops break down with overt B T malaria with almost mathematical certainty a few weeks after the cessation of atebrin suppression. It would be impossible to follow Dr MACDONALD's suggestion of taking men off atebrin in base areas

in New Guinea, since most of these troops are already infected with benign tertian malaria. Actually little notice is taken of a benign tertian attack except to treat it when it occurs. I entirely agree that malaria in base areas should be and can be controlled by preventive measures and that prevention is better than cure. I was glad to see that the experience of Brigadier FINDLAY and Professor MAEGRAITH confirmed ours in regard to the preference for atebrin estimations of plasma, and the fact that despite prolonged administration of the drug in their experience the level was maintained.

Major REID continues to use whole blood; this method is considered unsatisfactory by practically all research workers both in U.S.A. and U.K. and has generally been discarded. The important thing however with atebrin is not the blood or plasma level. The important thing is: Are these men fit enough to fight and keep on fighting for months in hyperendemic areas if they take atebrin (0.1 gramme daily) despite being repeatedly infected? I think the evidence is overwhelmingly in favour of the view that they can.

In regard to Brigadier FINDLAY's remarks about the sulphonamides, we found sulphadiazine and allied drugs effective in suppressing and curing most infections with P. falciparum but they failed absolutely with P. vivax infections. Personally I would prefer not to give gramme doses of any of these sulphonamides for a long period when other less toxic drugs were available. Sulphamerazine gave the most sustained concentration when given once in the 24 hours and for this reason might be preferred by some to other sulphonamides for prophylactic use.

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place,

on

Thursday, 15th February, 1945, at 3 p m

THE PRESIDENT

SIR HAROLD SCOTT, K.C.M.O., M.D, F.R.C.P, F R.S.E.,
in the Chair

PAPER

THE USE OF THE NEW INSECTICIDE DDT IN RELATION TO THE PROBLEMS OF TROPICAL MEDICINE.

BY

Professor P. A. BUXTON F.R.S.
London School of Hygiene and Tropical Medicine

CONTENTS

INTRODUCTORY

The ideal insecticide would possess a large number of different qualities. High among them is toxicity to insects of many types combined with safety when brought into contact with mammals and plants   Almost equally important, for most purposes is stability so that the material may continue to kill insects for the longest possible period in the place where it may have to work   it may therefore, be desirable that the insecticide should persist in the presence of sunlight, rain, growing plants the soil and so forth   There are other qualities almost equally desirable, for many purposes, e.g., lack of stain or offensive smell, absence of harmful effects on fabrics metals etc., ease of manufacture and low cost, and adaptability to many methods and purposes

Many years of research have been devoted to synthetic insecticides, with the hope of replacing the vegetable insecticides by substances capable of being manufactured as required and capable also of modification to meet particular requirements. It seems clear that the substance known as DDT (dichlorodiphenyl trichlorethane) is closer to the ideal than any other known insecticide. The insecticidal powers of this substance were discovered by the firm, J. R. Geigy A.G., of Basel, Switzerland their early patents protecting its use as an insecticide date from March 1940. Patents in many other countries have been applied for to cover the use of this and closely related substances.

It is common knowledge that DDT has already won a great name in military hygiene. There can be no doubt that its wide use in the tropics will be followed by a great reduction of harmful insects and will give us a more ready control of certain diseases carried by them but much remains to be done in exploring the potentialities of this substance. There can, therefore, be no doubt that it is desirable to bring out a statement of the present position and to discuss the possible uses of DDT. This I have found a most difficult and embarrassing task because the early work was confidential and had only a limited circulation. The majority of the authors have not yet had an opportunity of putting their work together and publishing it and I hope they will do so. In the meanwhile, in order to make this paper useful, it has been necessary to quote some of the unpublished British work, making acknowledgements to the numerous authors. I trust that they realize that no alternative was possible. As to unpublished American work, I have felt even greater hesitation in making use of it, and have not done so. But, though I have not quoted unpublished American work, all British workers, including myself, have been influenced by the very large amount that has been done and freely put at our disposal. It is satisfactory to say that there are extremely few points on which British and American workers differ so that my decision where work is unpublished, to confine myself to the British work has not greatly reduced the value of this paper.

This is a fitting opportunity to acknowledge much help and kindness which I received from American entomologists during an official mission to the United States on behalf of the Ministry of Production in the summer of 1943. A very free exchange of information on insecticides was arranged and has been in operation since. British entomologists have certainly benefited greatly from that exchange which has been most stimulating.

The British work has been carried out in a number of laboratories. It has been fostered and co-ordinated by the Insecticide Development Panel of the Ministry of Production. The chairman of that panel, Professor I. M. Heilbron, F.R.S., has recently published an interesting general review of the subject. (Heilbron 1945)

In a paper addressed to this Society one may be excused for omitting to discuss the application of DDT or other new insecticides to problems of the

farm and garden. The control of the insects of stored products is barely touched upon, and certain uses of DDT in military hygiene are also regarded as outside our scope.

There is another remarkable new insecticide benzene hexachloride, or 666. It is understood that a general statement about it is forthcoming in the near future, and we look forward to hearing more of it. Against certain insects the effective dose of the active isomer of benzene hexachloride is even less than that of DDT. It is being introduced by Imperial Chemical Industries, Ltd.

Owing to the urgent pressure of the war very considerable developments have been made with several groups of synthetic insecticides. Important advances have been made in the United States, Russia and Great Britain (and doubtless in Central Europe, too).

CHEMICAL CHARACTERISTICS

The initials DDT stand for dichlor-diphenyl-trichlorethane. This name is not completely specific, for it would cover several very closely related substances. If it is necessary to be precise the alternative lies between 2, 2 bis-(parachlorphenyl)-1, 1, 1 trichlorethane and alpha alpha-bis (p-chlorphenyl), beta beta beta-trichlorethane.
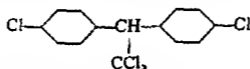
This may be expressed as $(C_6H_4Cl)_2 CH.CCl_3$.

Even those whose acquaintance with organic chemistry is that of the dilettante should remember that the substance is the para para compound and that if either of the end chlorines is moved into the ortho position the substance is almost harmless to insects.

The names "Gesarol" and "Neocid" are registered by the Geigy Company and refer to certain mixtures containing DDT prepared for use in horticulture, and the control of lice, etc. These names should not be used as they have been, for the active substance itself here referred to as DDT. The spelling "guessarol" is used in England in place of gesarol as the latter is very similar to the registered name of another article. For further proprietary names see WEST and CAMPBELL (1944).

The melting point of the pure para para para substances is 108° C., the molecular weight 354.5 and the density 1.6 gramme per ml. DDT is a white crystalline substance with a faint pleasant smell. It is exceedingly non-volatile at ordinary temperatures, so far as I know the vapour pressure has not been determined but it is very low indeed, a small fraction only of that of mercury. DDT dissolves in most organic solvents, the solubility in some of the commonest of these is given in Table I.

TABLE L

Approximate solubility of DDT in grammes of DDT per 100 ml. of solvent, at 27 to 30° C.

Cyclohexanone	100 to 120
Benzene	77 _ 83
Ortho dichlorbenzene	63 _ 71
Ethylene dichloride	56 _ 62
Xylene	56 _ 62
Acetone	50 _ 55
Carbon tetrachloride	46 _ 48
Methyl salicylate	39 _ 41
Benzyl benzoate	39 _ 41
Dimethyl phthalate	31 _ 33
Ether	27 _ 28
Ethylene glycol monoethyl ether ("Cellosolve")	17 _ 18
Pine oil (Hercules "Yarmor 302")	15
Diethylene glycol monoethyl ether ("Carbitol")	12
Sesame oil	10
Fuel oil, various	8 _ 10
Cotton seed oil	8
Kerosene, crude	8
Oleic acid	8
Castor oil	7
Kerosene refined	4
Ethyl alcohol, 95 per cent.	1.5

With regard to mineral oils, which are particularly interesting as they are so much used as solvents for insecticides, it is to be noted that DDT is more soluble in olefines and cyclic hydrocarbons than in paraffins. It tends, therefore, to be more soluble in the less highly refined oils, as a general rule. It should be remembered that DDT is heavy and addition to a crude oil might produce a solution so heavy that it would sink in water.

The solubility in water is very low but as the material is intensely insecticidal one cannot assume that a solution in water is harmless to such insects as mosquito larvae.

DDT is stable in the presence of light, ultra-violet water vapour and boiling water. DDT is without effect on metals, fabrics, leather and dyestuffs, though it will be remembered that as it is generally applied in organic solvents or emulsions, a spray containing DDT might be harmful to paints, varnishes, etc.

Present day commercial samples vary somewhat widely in their purity depending on the process used in manufacture and on whether the material has been recrystallized. much that is at present available has a purity of 60 to 70 per cent. The principal impurity (the ortho para compound) has been

isolated and shown to be only slightly insecticidal For accurate work therefore, one should either use chemically pure samples or state the amount of the pure para para compound in the material which was used. If that is not known it is worth while to record the setting point of the material used *i.e* the temperature at which the molten material begins to set solid in a test tube as it is gradually cooled in a water bath The setting point of the main impurity the ortho para compound is 82° C. For this reason the setting point of a commercial sample is always below that of the pure para para substance (108° C.) and, generally below the boiling point of water

A number of closely related polychlorethanes have been synthesized and tested on different insects by several different techniques. Several of them are insecticidal and in one or two the insecticidal efficiency approaches that of DDT (MARTIN STRINGER and WAIN 1943) None of these substances is available commercially The principal source of information on the chemistry of DDT and related substances is the paper of LAÜGER MARTIN and MÜLLER, 1944)

No information has been disclosed as to the cost of production of DDT or the price at which the material may later be marketed According to CAMPBELL and WEST (1944a), American production had reached 300 000 lb per month when their paper was published in September 1944 and a much higher figure was aimed at. British figures are not generally available DDT is not at present on sale in Britain.

TOXICITY OF DDT TO MAMMALS

An important consideration is that DDT has very little smell and as it is nearly insoluble in water it is tasteless. One might, therefore swallow relatively large quantities over a period of time (if, for instance, one was eating food which had been sprayed) and there is the possibility that this might lead to chronic poisoning Furthermore, though the solubility in water is so low the substance is soluble in fats and cooking oils and might then be absorbed and the stability of DDT in a chemical sense might possibly lead to chronic or cumulative effects. It has also to be remembered that DDT is used as an insecticide in many ways so that there are several ports of entry into the human body

The toxicology of the substance has been fully investigated by Professor G R. CAMERON in this country and by at least three groups of workers in the U.S.A. It appears that none of the British work has been published

In reading the toxicologist's results it has to be remembered that it is his purpose and duty to introduce the material into the animals body and study its effects To do this he may carry out some procedure (such as injection into the peritoneum) which seems remote from the practical issue. One cannot criticize the toxicologist for such experiments if they assist his work but on the other hand the potential user must relate the toxicological facts to his work and problems. In doing so he may require to exercise robust common sense.

The most important toxicological facts appear to be as follows —

1   *Effect of dry DDT on the skin.*—Solid DDT (5 per cent.) ground with a mineral diluent appears to be harmless on the skin of laboratory animals whether put on dry or as a paste with water (DRAIZE, NELSON and CALVERT 1944). It does not retard the healing of cuts or abrasions (CAMERON M.S.)

2   *Effect of solutions on the skin.*—Experiments are reported in which the fur of a rabbit is clipped and a soft rubber cuff (tight at the ends, slack in the middle) slipped over the trunk, the dose of DDT in a solvent being introduced under the cuff. Under these conditions, 75 per cent. or more of the dose of dimethyl phthalate or dibutyl phthalate is absorbed. Giving DDT at 25 to 30 per cent. in either phthalate on a single exposure lasting 24 hours symptoms of DDT poisoning were invariably seen, though no deaths occurred the doses ranged from 3·9 to 9·4 c.c. per kg (approximately 1 to 2·8 grammes per kg)   In other experiments, 30 per cent DDT in dimethyl phthalate was rubbed into the skin of experimental animals, daily for 90 days. In rats, rabbits, and guinea-pigs at daily doses from 150 to 1,200 mg. per kg symptoms were invariable and often observed after the first inunction. the characteristic loss of appetite leads to poor health and death from secondary infections later in the period in some individuals of each species but in dogs at the same daily dose no symptoms occurred (DRAIZE, NELSON and CALVERT 1944) SMITH and STOHLMAN (1944) report that a solution of DDT in dimethyl phthalate painted daily on the skin for 12 days produces nervous symptoms at a lower dose 100 mg per kg

As dibutyl and dimethyl phthalates are used as repellents against biting insects, CAMERON and BURGESS have examined the risk that in their presence DDT might be particularly readily absorbed and poisonous they conclude that these phthalates do not increase the absorption of DDT in comparison with other solvents (ether kerosene), in which it has been applied to skin of rabbits.

There are no records of dermatitis from DDT

Sensitisation is, apparently uncommon following the application of DDT to the skin. DRAIZE, NELSON and CALVERT report mild but definite sensitisation in guinea-pigs, to which the Landsteiner technique was applied.

3   *Effect on eyes* —There is no evidence that DDT is harmful to the eye, including the conjunctiva. Ointments and colloidal solutions containing up to 5 per cent. have been put on the conjunctiva without ill effect (WARRICK and UNTI, 1944).

4   *Effect on respiratory system* —It was evident from the first that danger from sprays and dusts was one of the things which must be studied. An investigation on the effect of aerosols mists and dusts containing DDT was started in Washington by a group of well-known toxicologists in the summer of 1943, and results have recently been reported (NEAL, von OETTINGEN and others.

1944). A very large amount of work has been done and the results are fully set out. The authors made use of mists and aerosols containing a variety of solvents, and of dusts, some of them undiluted, others containing about the proportion of DDT which is used in practice. The experimental animals (and men) were in closed chambers and in some cases were exposed daily for periods of weeks. The initial dosages were measured, but as most of the particles settle quickly the concentrations could not be maintained. Several sorts of experimental animal were used including a few monkeys and two men. The men were very carefully examined before and after exposure in addition to a general physical examination, the reflexes and manual steadiness were tested and full analyses made of urine and blood. The men were also subjected to a " battery of psychophysiological tests ". The changes observed after exposure to the DDT are set down in detail; they are slight. The authors conclusions are that —

" The experiments described in this report allow the following conclusions —

In spite of the inherent toxicity of DDT its use in a 1 to 5 per cent. solution in 10 per cent. cyclohexanone with 89 or 85 per cent. of Freon as aerosol should offer no serious health hazards when used under conditions such as those required for its use as an insecticide. It should be pointed out that the solution of DDT in fatty oils definitely increases its toxicity and that the results obtained by using a solution of DDT in cyclohexanone are not necessarily comparable to the effects produced by a solution in oil.

The use of DDT in concentrations up to 10 per cent. in inert powders, for dusting clothes, as in the extermination of lice, appears to offer no serious hazards because of the relative insolubility of DDT and the large particle size of the dust. Therefore, it does not reach the alveolar spaces. A large proportion of the dust is retained in the upper most sections of the respiratory tract. The remainder is swallowed. On account of its relative insolubility it is thought that only a small fraction is absorbed.

Because the use of a 1 per cent. DDT-deobase mixture was found to be non toxic to rabbits with heavy exposure for 48 minutes daily over a period of 4 weeks, it is believed that its use as a fly spray which involves only temporary and comparatively moderate exposure to much lower concentrations, should be safe. However due to the fat-solvent properties of most petroleum distillates, irritation of the skin may occur following heavy exposure.

Although this study deals only with the appraisal of the potential dangers of DDT when inhaled as aerosol, dust, and mist, it should be pointed out that ingestion of massive doses of DDT will cause a toxic reaction. It should, therefore, only be used under conditions which exclude the heavy contamination of food.

Since these experiments were concluded a thorough clinical and laboratory study has been made of three men who have each had several months continuous occupational exposure to DDT used in various forms as an insecticidal agent.

An evaluation of the results of these examinations fails to indicate any definite evidence of toxic effects from the exposure the three subjects have had to DDT

5 *Effect on digestive tract* —Work is reported in which DDT has been administered by the mouth in corn oil in a single dose. The number of animals was not great, but the figures indicate the following approximate median lethal dose (in mg per kg) rat, 200. mouse, 400 to 500· rabbit, 400 or over guineapig 250 to 600· chick over 300 (no deaths in five chicks at this dose) (WOODARD NELSON and CALVERY, 1944) The same authors also report experiments in which dry powdered DDT was added to the rations of rats, daily. A daily dose of 0·10 per cent. resulted in some deaths after a few days other rats

showed symptoms, but later recovered a few living for as long as a year in spite of the dose being given daily for that period. In guineapigs on the same doses the effects were rather less. Chicks were killed in 4 to 16 days, on 0.05 per cent.

Another group of American workers find that in a single dose, in olive oil, the median lethal dose for rats is 150 and for rabbits 300 mg per kg. At about one-third of these doses the animals are abnormally excitable and may have mild tremors. Repeated doses of 50 mg DDT per kg in olive oil are lethal to rabbits, if given daily for 15 in 23 days (SMITH and STOHLMAN, 1944).

There is unpublished British work, showing very similar median lethal doses in a fat solvent, to produce serious symptoms with an emulsion of DDT in gum arabic much larger doses must be given.

Several of the toxicologists have commented on the variability of their results. Whether this is due to irregularity in absorption, or of metabolism, is not known in either case it may result, justifiably in hesitation in declaring that some particular dose or exposure is safe.

It seems remarkable that none of the published papers on the toxicology of DDT states the degree of purity of the material used, or appears to give consideration to any of the impurities which occur in the commercial product.

The onset of symptoms, in an experimental animal is never rapid. A common early symptom is abnormal excitability with fine tremors, and loss of appetite (the last being perhaps the most easily detected symptom if animals are given repeated small doses) recovery at this stage seems common. If larger quantities of DDT are given the tremors become more pronounced, with flaccid or spastic paralysis and convulsions death is generally due to respiratory failure (SMITH and STOHLMAN 1944).

In postmortems of animals which have died of DDT poisoning, or received large doses (single or repeated), changes in the cells of the central nervous system are inconsiderable though vacuolation occurs round large nerve cells in the cord and cerebral motor nuclei in animals of several species. There are striking and elaborate changes in the liver particularly severe after repeated administration of DDT for large doses may kill so quickly as to prevent the development of obvious damage in the liver. Large areas of focal necrosis or zones of centrolobular necrosis are seen throughout the organ, indicating that many liver cells are destroyed. Phagocytic cells enter these areas and together with autolysis lead to the removal of dead tissue. Bile ducts are not affected. Provided life can be maintained over a critical period of impaired liver function, repair will set in and complete regeneration of the liver follow if secondary infection can be avoided. Fibrosis of the liver is seldom seen even with repeated applications of huge doses of DDT.

Other organs seem to be little affected. Sometimes there is slight damage to kidneys, especially if liver function is extensive, but functional disturbance

damage

is not a prominent feature Occasionally the heart muscle and the adrenal cortex are involved in focal damage but these lesions are not important (LILLIE and SMITH 1944). It should be emphasized that all these changes are the result of continued exposure to large doses of DDT experimental animals tolerate amounts of an order well above that to which man is exposed and show no evidence of injurious effects Pathological lesions are also described by NEAL, VON OETTINGEN and others (1944).

SMITH and STOHLMAN (1944) describe a method of estimating DDT in tissues fluids and excreta. They have found the drug in organs and fluids of animals poisoned by it.

To sum up the toxicological evidence on experimental animals it indicates that there is no risk from the dry material or watery suspensions, on the skin or swallowed. In the presence of solvents (including edible fats) DDT is absorbed through skin or from alimentary canal Large doses give rise to symptoms of which tremors are early (and manual steadiness has been suggested as a test in suspected DDT poisoning) After symptoms and liver changes have developed animals (and presumably men) recover if they cease taking DDT

I conclude that the toxicological work indicates where the risk might lie, but does not suggest that the material, used as an insecticide, is harmful. In this connection one may quote the words of one American group of toxicologists. "No irritation was noted from powdered DDT when applied by patch tests or on the hands of operators who have had almost daily contact with it during the past year Various toxicological experiments with eighteen preparations of DDT indicate that DDT in the solid form appears safe for use in preparations intended for topical application to the skin. From solutions DDT is absorbed and is a severe systemic poison however a number of preparations submitted to us containing DDT in concentrations up to 5 per cent. have proven safe for limited use." (DRAIZE, NELSON and CALVERT 1944).

One may turn to the experience of those who handle DDT in manufacture and in use. There are men who make and handle the raw material, at the rate of tons per week. Others have produced concentrates (20 to 30 per cent) in considerable quantity or impregnated very large numbers of shirts from solutions such men wear protective clothing There are also entomologists who have handled and distributed DDT for a period of nearly two years (and longer on the Continent) in many forms as solutions emulsions dusts and so forth Tons of dust (5 to 10 per cent.) have been distributed under clothes, and some hundreds of thousands of men have worn impregnated shirts In addition a small number of factory hands and entomologists after considerable exposure, have been carefully watched by physicians, whose examination has included biochemical work on blood the functions of the liver and so forth.

In all this varied, practical experience on human beings some of them ignorant and careless men, no harmful symptoms of any sort have been

penicillin of insecticides (a phrase of more glitter than clarity) and think that it will suffice to get some and ship it overseas. That is very far from the case, and the medical reader will hardly have to be reminded of the vast amount of careful investigation that has been needed in order to exploit the properties of penicillin or any other valuable drug. So it will be with the new insecticides of which DDT seems the most valuable. We shall not benefit from it unless we apply it with brains adapting its use to the habits of particular insects. Much research remains to be done, some of it fundamental and most difficult, much of it technological. It will be a long time before we can apply DDT neatly, economically and effectively.

1. MOSQUITOES.

Adult Mosquitoes. Sprays.—Owing to the shortage of pyrethrum and the immense new demands arising from tropical fronts, much work was devoted to the study of adjuvants and activators, in the hope of extending the available supplies of pyrethrum extracts and making them more effective. That work has become less urgent since DDT became available, and most sprays for use against mosquitoes or flies now rely on DDT to kill the insect, some pyrethrum extract being added if a quick knockdown is desired. The British official formulae are given below under House Fly (p. 382).

In considering doses of spray it is an error to suppose that mosquitoes are much more easily killed than house flies (as they are by pyrethrum sprays). There is evidence that *Aedes* at least more closely approaches the house fly in resistance to DDT as a spray.

A number of new and ingenious devices have been developed for dispersing insecticidal sprays and mists. The so-called insecticidal bomb has been referred to in print though no full account of it appears to be available. Several types are in use of which that made by the Westinghouse Company was probably the first in the field. The general principle is that of a soda-water syphon, the liquid insecticide being at the bottom, and a compressed gas (generally "freon," or dichloro difluoro methane) above it: a capillary dip tube passes down the inside of the bomb into the insecticide, and at the top of the dip tube is a fine nozzle and release mechanism. When the release is opened the freon drives the insecticide up the dip tube and out of the nozzle. The pressure and fineness of tube and nozzle ensure that the spray is delivered in very minute droplets, as an aerosol, or mist, the freon which was liquid under pressure in the bomb having become a gas on release.

The mist has remarkable powers of penetration into small refuges in which insects shelter, and if used with skill and restraint is economical of insecticide. Most of the bombs so far available have contained pyrethrum insecticides, but it is probable that similar devices for dispersing DDT may be contemplated.

GOODHUE (1944) has provided a most useful account of recent American work on the distribution of insecticides as aerosols

A disadvantage of any apparatus which distributes aerosols is that they are so economical, and the mist so fine that no appreciable film is built up In this respect some good type of hand or power sprayer is preferable, for it is more adaptable and can be used for spraying insects in a room or for depositing a film of DDT

Adult Mosquitoes Films —The reduction of *Anopheles* by spraying houses with pyrethrum is now a well-established method of controlling malaria. In spite of the need for reapplication about once a week it has been shown to be almost the only effective measure capable of being paid for by a tropical village community Application of DDT is more effective because so much more lasting and there is evidence from several parts of the world that it very greatly reduces the number of mosquitoes in houses and that the numbers are often held down to a very low figure for 2 or 3 months after a single spraying The reader must, for the moment accept an assurance that this is so he will join with me in hoping that the large amount of solid work which has been carried out will soon be published For the moment a dosage of 100 mg per square foot should be aimed at put on as described under *Cimex* But in view of the high susceptibility of mosquitoes (or rather of the few species which have been tested) to DDT it seems likely that lower dosages may later be found successful.* Much detailed research is also required on the entomological side to discover just what type of resting place is most frequented by mosquitoes of particular species Further work may well show that such qualities as roughness colour darkness and so forth decide where the insect will rest, and it may prove economical to provide attractive refuges or areas of wall and confine the DDT spraying to them

The properties of films of DDT will also compel us to review what we know of the house-haunting habits of different species of *Anopheles* and indeed of insects generally If a mosquito enters a house and rests on a wall before attacking man it might well pick up enough DDT to prevent its biting We understand that this actually happens in parts of India and that what the ordinary man notices is absence of annoyance from mosquitoes this is consistent with an observation by DAVID and BRACEY that *Aedes* sprayed with a dose of DDT which would later kill them might be able to fly but did not bite the experimenters A female of another species may enter the house and at once feed on man subsequently settling on the treated wall In that event though the man has been bitten the community will be well protected from malaria, because few mosquitoes having these habits will survive long enough to bite a second time. Individuals of yet a third species may hardly rest in the house,

* The figures quoted below by MACDONALD (page 393) are much lower They are expressed in mg per sq metre To convert to sq feet, in conformity with this paper divide by 10·6

simply entering, feeding and leaving more or less directly Anopheles punctulatus and A maculatus are examples. It may well be that DDT applied as a residual film in houses will prove of little value against insects with these habits. Enough has been said to indicate the importance of the subject.

Another promising use of the film effect is by the impregnation of relatively wide-meshed bed nets, which would probably make them effective barriers against small species of Anopheles and also against such little pests as Phlebotomus

It seems probable that the use of DDT films would solve the problem of insect transmission by planes, and prove to be an extremely simple and effective " anti-amaryl " measure The plane could be treated inside at its regular overhauls, and this would do away with the present necessity of spraying it whenever it lands in certain parts of the world

Mosquito larvae Dusts —DDT powder is not easily wetted though considerably heavier than water and there were good prima facie grounds for hoping that it might be an excellent dust for the destruction of *Anopheles* larvae Laboratory tests gave promise of brilliant success, and it may yet be possible to produce commercially a floating unwettable powder free running, and containing a sufficiency of DDT One difficulty is that DDT alone is difficult to grind to a fine powder It must therefore, be ground with a mineral, and transported as a 10 per cent powder from the works to the field. The transport problem, therefore presents itself once again. Field tests so far have appeared disappointing and the matter seems to have passed into the background as entomologists of several countries pressed on with work which seemed more urgent, or more immediately promising

Mosquito larvae Oils —In contrast to dusts the use of DDT in larvicidal oils is evidently an immense practical advance In the past it has been necessary to use 10 to 20 gallons of a suitable oil per acre this may now be reduced to less than a gallon, with enormous reduction in transport, and in the costs and difficulties of distribution.

The dosage to be used depends, of course, on circumstances. A standard figure for many types of water is 1 to 2 imperial quarts per acre of a 5 per cent (w/v) solution of DDT in an oil of good spreading power Remembering that one imperial quart equals approximately one litre one can see that one quart per acre of 5 per cent. solution is about 50 grammes or rather under 2 ounces of solid DDT (making no correction for the impurity of the sample of DDT). It is often very difficult to put down so small a volume as one or two quarts on an acre, even using a fine nozzle and low pressure on the sprayer it may be preferable to double or treble the volume of oil, using the same amount of DDT

In some early reports on DDT in oil as a larvicide it was suggested that

the DDT is a spread-aider. This is known not to be the case, though some commercial samples of DDT contain an impurity (probably ethyl alcohol) which causes an oil solution to disrupt violently and disperse itself widely over a water surface. This impurity is not generally present and one must for the moment use oils which themselves spread satisfactorily. A development of the near future will be greater attention to the spreading pressure of oils used as larvicides (with or without DDT) and to the strength of the natural films which occur on all natural still waters, however clean they may appear and which offer resistance to the spread of oils over the surface.

Reports are conflicting as to the lasting effects on natural waters of films of DDT in oil at one or two quarts of 5 per cent. per acre or in larger doses. The cause of the discrepancies is unknown. The problem is an important one, for local study.

The above dose (1 to 2 quarts) well distributed is effective against mosquito larvae of many kinds probably because the quantity of DDT is greatly in excess of what would be required if distribution were perfect. I have recently had an opportunity of defining the amount of DDT in oil, which would kill larvae of *Anopheles funestus* in ditches in the Gold Coast. As my purpose was to compare different solutions and emulsions I was interested in defining the minimal dose at which the materials began to fail, and I took particular care to disperse my material over the small areas of water on which I was experimenting. It must, therefore, be clearly understood that my conditions were far from those which prevail in practical control problems. Working with DDT in a diesel oil which spread well I found that 0.10 c.c per square yard of a solution containing 5 per cent. of pure DDT killed all larvae and continued to kill for some 4 days (except that an occasional first instar larva appeared before that, probably destined to die). This dose is extremely small, corresponding to 2.4 grammes or 0.8 ounces per acre, a figure well below the conventional 2 to 4 ounces (corresponding to 1 or 2 imperial quarts) even allowing for the fact that my material was pure para para DDT. Even more remarkable, a tenth of this dose (0.01 c.c per square yard or 0.08 ounce per acre) killed all larvae but had no lasting effect.

An interesting and puzzling observation was that doses of 0.10 c.c or over gave a complete abolition of larvae for some days followed by partial control for a long period. For instance I selected a roadside ditch permanently full of water and with a rich flora of sedges and of submerged algae. It was treated with 0.20 c.c of above solution per square yard, and gave a complete kill for 6 days followed by a great reduction in larvae prevailing at least to the end of the 4th week. Other examples of similar imperfect control were observed (BUXTON, 1945). It is most difficult to understand how DDT carefully and uniformly applied to a small area can last sufficiently to kill most larvae but yet let a proportion grow up.

Mosquito larvae   Dispersions.—It has been shown that DDT is poisonous to mosquito larvae if it is dispersed in a colloidal form through water one part in ten millions killing larvae of *Anopheles albetarsis A strodes* and *Culex* in under an hour one part in fifty millions in 24 to 36 hours, in an aquarium moreover the water remains larvicidal for months. Work of a similar sort has been carried out with remarkable success in a swimming bath. (WADLEY and UNTI 1944) It is not clear what means were used for obtaining the above dispersion at these extremely low doses it seems probable that all DDT was in solution There is much unpublished work which, in general, supports the conclusions of these authors.

It is not yet clear that this method will have much general value. There may be peculiar situations in which the use of DDT in oil films is undesirable, but in general they seem preferable. Under most circumstances dispersion through the depth of the water seems unnecessary because it would appear to waste DDT (a film on the surface being sufficient) and because it may kill submerged insect larvae which are valuable as predators on mosquito larvae. It is possible that these dispersions may give us a method of killing the larvae and pupae of *Simulium*, which are so difficult to attack as they live completely submerged in running water The matter should be approached with caution, for the use of a dispersion or solution of DDT in the water might do much harm, either to the natural enemies of *Simulium* or to fish food, or to fish.

Distribution by Plane.—The use of aeroplanes for the distribution of DDT in oil was originally regarded as a larvicidal measure and there is evidence that it is very effective especially on large bodies of water But it is found that some of the oily spray contaminates surfaces and kills many adult mosquitoes. Moreover it does not only kill day biting mosquitoes which might impact on spray particles while in flight, but it also kills night biters (*Anopheles*) which presumably pick up DDT from surfaces on which they alight during the night after the spraying

General Effects on Malaria.—It is clear that DDT is of enormous value, for the destruction of adult and larval *Anopheles* and it is quite possible that it may have a major effect on malaria in villages, which is hardly touched by existing methods. This will have very far reaching effects on population and indirectly on agriculture, land hunger erosion, and many economic problems.

2. HOUSE FLY (*Musca*).

Adult flies Sprays.—It seems that there is little published information on which one could precisely evaluate DDT as a spray against adult house flies. GRINDORY and McGOVRAN (1944) compared a good commercial sample of DDT (setting point 91 C) with certain other insecticides using the turntable method. A concentration of 2 5 grammes per litre in kerosene gave 100 per cent. kill and half this figure did not. They also showed that the following combinations were effective against adult *Muscas* —

DDT 0·10 per cent. + pyrethrins 0·03 per cent
      0·05   +   0·05
      0·10   + thanite   2·0
      0·10   + lethane 384 2·0

The above mixtures gave a very high knockdown in 10 minutes or less as well as a kill of 90 to 100 per cent. Used alone 0·10 per cent. DDT gave no knockdown and 80 per cent. kill

Unpublished figures by PARKIN and GREEN of the Department of Scientific and Industrial Research show that 0·05 per cent. DDT (w/v) plus 0·02 per cent. pyrethrins is barely satisfactory against flies These workers then define the knockdown and kill of a mixture containing 0·10 per cent. DDT with 0·03 per cent. pyrethrins tested in a chamber They then sprayed this at 2½ c.c. per 1 000 cubic feet in rooms and army huts at about 24° C using hand sprayers and confirmed that the mixture gave a high knockdown and killed all the flies. They have also shown that mixtures of DDT and pyrethrum in kerosene can be stored under suitable conditions for at least 17 months at 27·5 C without marked deterioration.

The present British official recommendation for a general spray for killing adult flies and mosquitoes is —

          0·07 per cent. pyrethrins (or more if available)
    or 0·05   + 0·3 per cent DDT
    or 0·03   + 0·5

In these figures the percentage DDT refers to the pure para para substance The dosage is 10 c.c per 1 000 cubic feet (1 fluid ounce per 3 000 cubic feet) subject to considerable latitude if used in rather open huts and so forth. There is very little to choose in effectiveness and the formulae allow considerable freedom, depending on supplies

Adult flies   Films —Residual films will probably prove even more valuable in fly control than are sprays On the laboratory side BUXVINE's figures quoted above show that *Musca* is susceptible to traces of dry DDT in the surface on which it settles WIESMANN (1943) showed that a very small dose of spray containing DDT allowed to dry on glass killed flies (*Musca* and *Stomoxys*) which walked on it even for 30 seconds The deposit on glass retained its potency for 3 months or more He sprayed walls and ceiling of a cowshed in 1942. In the shed there were enormous numbers of *Musca* and *Stomoxys* and the cows were very restless He found that two sprayings (dose per unit area not stated) would almost eliminate flies for one season LINDQUIST MADDEN WILSON and JONES (1944) applied DDT in a number of different solvents on the inside of unpainted wooden cages at 25 mg DDT per square foot. The period necessary to paralyze all flies became longer as the days passed for instance, with kerosene as solvent the period was 15 minutes after 15 days

90 minutes after 45 days, 220 minutes after 100 days, and so forth. They found that with any solvent this dose of DDT killed all flies even as late as 265 days after application. They observed that on painted surfaces (and particularly on those freshly painted) DDT lost its efficacy more quickly than on plain wood, presumably because the solvent carried some DDT into the paint. To judge from these figures, it seems that a film which would be inadequate to kill bugs may be extremely effective against flies.

This is supported by ample evidence from several parts of the world. It now seems that one application will keep a cowshed clear of flies for the duration of a European or North American summer. The method is applicable to restaurants, markets, latrine screens, and indeed to almost any surface on which flies settle. A very minute dose is effective on glass, presumably because the DDT crystals are readily detachable, and all on the actual surface; one may therefore put an invisible film on the inner surface of a window and be free of the buzzing of insects for weeks. LADGER and others (1944) state that the minimum dose fatal to a fly applied on glass, is 10^{-5} or 10^{-6} micrograms of DDT per sq. cm. (a microgram being a thousandth of a milligram, or millionth of a gramme). This corresponds to about 10 molecules of DDT per sq. micron.

3. OTHER INSECTS

There is evidence that many adult muscids (*Calliphora, Lucilia, Chrysomyia, Stomoxys*) are very susceptible to DDT in the form of a residual film. The material may therefore prove very valuable in tropical slaughter houses, meat markets and so forth. It may also be sprayed (from kerosene or emulsion) on animals to kill *Stomoxys* and other biting flies which attack them. I have seen an extraordinary reduction of *Stomoxys* and haematophagous *Musca sp.* in West Africa following the spraying of one cow out of a small herd of a dozen animals which were suffering very greatly. Sprays and emulsions of DDT have also been applied to cattle in Texas (WELLS 1944). This author records that using an aerosol containing 5 per cent. of DDT (the dose per cow or per unit area not being stated) he obtained an almost complete control of *Lyperosia* (*Siphona*) *irritans* for some 2 weeks even if he only sprayed some of the cattle and them only on the back. He makes the interesting observation that crystals of DDT easily break off cow's hair and proposes to test the effect of stickers. Using an emulsion and a power sprayer he found that if 2 to 3 pints containing 0·2 per cent. DDT were applied per cow he obtained almost complete freedom for a week, and a reduction to one third after 2 weeks. It may be remarked that on the second occasion the insecticide may still have been effective though acting slowly.

Provisional work by NASH and myself has established that *Glossina spp.* are very readily killed by traces of DDT on cloth. This opens up great possibilities of control, by treating bait animals with DDT emulsions, or by

impregnating clothing or sacking screens. The matter will shortly be more fully investigated.

Fly larvae—Work on DDT against larvae of *Musca domestica* breeding in manure heaps in winter in Florida is reported by SIMMONS and WRIGHT (1944) They made use of an emulsion diluted on the spot so that they could apply a large bulk to the surface of the manure they applied 0·6 U S gallons per cubic foot of manure, and obtained satisfactory destruction of larvae even at 0 1 per cent. DDT their lowest concentration Preliminary work on refuse in which *Stomoxys* was breeding was also successful

Nothing appears to be published of the stability of DDT in manure soil or similar materials This is a matter of great importance in medical entomology (in relation to early stages of many muscids also *Phlebotomus Culicoides* etc.)

4 HEAD AND BODY LOUSE (*Pediculus humanus*)

Persistent Insecticides—In dealing with an outbreak of lice on a large number of human beings the essential point is to use some insecticide which has a lasting effect. Unless one has such a material the clean people are extremely liable to become reinfested from the others or from stray lice on bedding clothes etc. despite organization discipline, etc. None of the methods in use early in the war (heat fumigants volatile materials such as naphthalene) was satisfactory in this respect, for none lasted for any considerable time. I realized that it is essential to use lasting insecticides for this purpose and began searching for them in 1939 The thiocyanates (in particular Lethane 384 Special) proved valuable and are now in current use in Britain against the head louse (BUSVINE and BUXTON 1942) They have also been used against body lice with great success on many thousands of Arab and Persian labourers but they tend to irritate the skin on the more delicate parts of the trunk and it is doubtful if Europeans would tolerate them, unless there was a serious threat of typhus. For further data see BUSVINE (1945). DDT applied in several ways, has proved invaluable against body lice, and is without doubt the most effective insecticide for this purpose because of its lasting powers

The Geigy Company in Switzerland were the first to discover the value of preparations containing DDT for the control of head and body lice, and I have seen their advertisements, dating from the latter part of 1942. We in London also discovered that DDT is very effective against lice BUSVINE's experiments in the early months of 1943 indicated a toxicity to lice about ten times that of the thiocyanates

Dusts—In the meantime the entomologists of the U.S Department of Agriculture at Orlando Florida, were carrying out tests with commercial dusts and other preparations of DDT Their method of testing which I was privileged to see in April 1943 was practical. A cloth sleeve was slipped over the arm or leg of an experimental subject, lice and powder introduced

into it, and the ends fixed to the skin above and below with adhesive tape. Results were examined after 24 or 48 hours if all lice were dead, more were introduced and the experiment continued (sleeve and powder remaining in position) till insecticidal action became very weak. DDT showed itself exceedingly potent, and much more lasting than other materials, and further tests were carried out in which men's underclothes were dusted and then infested with several hundred lice Infestation was repeated till the material began to fail (BUTHLAND MCALISTER, EDDY and JONES, 1944). This type of test is extremely practical. It appears to me after considerable experience, that the personal habits of the subject such as his restlessness or the way he dresses greatly affect the loss of powder from his clothes. It is, therefore, impossible by this method, to make a precise evaluation of two rather similar powders, and it may be doubted whether the difference in duration between a 5 per cent and a 10 per cent. DDT in mineral diluent can be certainly established

Mainly as a result of the work at Orlando a 10 per cent. DDT dusts now in very wide use It remains effective for 2 or even 3 weeks, and may kill lice after that assuming that the subject does not wash his garments. For men in winter clothes 11 ounces per treatment suffices.

Dusts are also effective against head lice and crab lice (*Phthirus pubis*). They are not ovicidal, but the DDT generally persists long enough to kill all the small lice as they emerge from the eggs.

It is generally known that very wide use was made of DDT dust in the control of the epidemic of typhus in Naples early in 1944 It is unfortunate that no scientific account of what was probably a remarkable piece of preventive medicine has yet been published. It is, however known that typhus had broken out, and might well have become a great and spreading epidemic, in view of the very unhygienic state of the people thousands of whom were living in crowded shelters. The method of application of the dust was by hand blowers, dust being puffed up the sleeves and trouser legs, down necks and into the waists of skirts and trousers This method is very quick, and acceptable to both men and women. Early in the epidemic a pyrethrum powder was used, as I understand and was proving successful (though one would not expect it to continue active for more than a few days). The population submitted to treatment partly because they were frightened by the threat of typhus, and because steps were taken to inform and persuade them by pulpit, press and radio but it is understood that there was also a considerable degree of compulsion, especially in parts of the city where typhus cases were found. What happened in Naples has been described as " the only completely proven victory which can as yet be inscribed on DDT's battle honours " It seems grudging and is no doubt useless, to point out that no one ever knows what would have been the course of an epidemic had certain measures not been taken that perhaps any good powder applied in this simple and ingenious

way would have proved effective and that the element of compulsion may have been a good second to the insecticide

Impregnation—An even more effective way of using DDT in the control of lice is by impregnating garments which then become insecticidal and capable of killing lice even after wear for several weeks and several washes in hot soap and water The early work is dealt with in the paper by BUSHLAND and others quoted above. It is perhaps sufficient to say that an addition of 1 or 2 per cent. by weight of DDT is all that is necessary This can be added to fabrics (cotton or wool) either from solutions in volatile solvents or from emulsions. For small scale work under field conditions the emulsions would be particularly valuable for they only require diluting to a particular figure after which a few garments can be ' louse proofed in a bucket. The method may therefore, prove of great value to explorers anthropologists and others whose lot it may be to live in close contact with the louse The application of the method on a large scale may also be of great importance in post war Europe, or in tropical areas faced with epidemics of louse borne relapsing fever A large amount of technological work has been done on impregnation and ample field tests have been carried out in different areas and under different conditions of climate.

A small amount of work has been carried out in Britain using emulsions to impregnate the hair of the head Dr J R. BUSVINE is good enough to let me say that a dose of 0·2 gramme of DDT completely proofs the head for a week but after a fortnight is beginning to fail in some cases.

One observes that goats have been washed with emulsions containing 0·07 to 0·6 per cent. DDT which killed all lice (Anoplura and Mallophaga) No lice were found nearly a month later but whether there had been an opportunity for reinfestation is not stated (BABCOCK, 1944)

5 BED BUGS (*Cimex*)

A population of bugs is so well concealed, at least by daytime, that one must employ either a fumigant or some contact insecticide which can be relied on to leave a lasting insecticidal film. Pyrethrum solutions in non-volatile oils are very toxic to bugs but not sufficiently stable to be satisfactory Thiocyanate insecticides (lauryl thiocyanate, the lethanes) are more lasting, but being oily liquids and administered in mineral oils are absorbed into plaster wood, etc. so that a large part of the dose is ineffective DDT can be put down in such a way that a considerable part of it remains as a solid lasting film on the surface. It is without doubt the most effective material known for bug control. If it is used intelligently by the community not by the individual housewife, the widespread infestation of urban areas should come to an end quickly

The earliest publication dealing with the effect of DDT on *Cimex* seems to be that of MADDEN LINDQUIST and KNIPLING (1944) Their earlier experiments satisfied them that very small quantities of DDT (from solvents or as

,dusts) killed bugs and remained effective for a long time. They then made a cage of unpainted wood, and demonstrated that a deposit of 100 to 150 mg. per square foot left by a spray would kill bugs on a 48 hour exposure at least as late as 10 weeks after the spraying. The same dosage was resistant to scrubbing with hot soap and water. They carried out successful small scale trials on bedsteads and in barracks.

BUSVINE's data quoted above have indicated that, on dry impregnated paper the dose of DDT which kills the bug and the louse does not differ greatly both insects are much more resistant than flies or mosquitoes. He allows me to add that the concentrations in oil solution that are lethal when sprayed directly on to bugs or lice are also not greatly different. In a series of experiments he deposited spray at 0.35 mg per sq. cm. on insects in Petri dishes. When the concentration of DDT in the spray was 0·34 per cent. this killed about 50 per cent. of lice the corresponding concentration for bugs being 0·56 per cent. These figures show that, tested in this way DDT is between five and ten times as toxic as the most effective synthetic insecticide previously known. It is in fact the first synthetic compound of which the insecticidal power approaches that of the pyrethrins or rotenone.

The unpublished work of my colleague Mrs BARNES makes our information on film action more precise. Using a Potter tower she put a range of doses of pure para para DDT on a number of types of surface (e.g., plaster painted wood, etc.) choosing such materials as are used inside houses. Bugs were kept on the surfaces for 24 hours at room temperature transferred to clean tubes, and examined for mortality 6 days later. To secure a kill of 100 per cent. a deposit of about 0·2 mg. per sq cm. (180 mg per square foot) was necessary if the surface was tested a few days after spraying. This dose was effective on keene's cement, unpainted wood and wood which had been painted some time before. When these surfaces were tested 1 and 3 months after spraying the kill was still 80 to 40 per cent. after 6 months it was 60 per cent. on the first two surfaces but very low on the painted wood. At lower deposits of DDT kill was never quite 100 per cent., and persistence was less. On glass, owing to none of the DDT being absorbed, 0·06 mg per sq cm (60 mg. per square foot) was 100 per cent. effective even 3 months after spraying. Mrs. BARNES finds that on smooth surfaces the heavier deposits of DDT form crystals large enough to be seen, and these are easily rubbed off in ordinary domestic dusting. Many other problems of a technological nature are encountered in her work. Adult bugs are more resistant to DDT than nymphs. Her work does not altogether support the rather far reaching claims based on the preliminary work done elsewhere (BARNES, 1945 a, b).

The active isomer of benzene hexachloride sprayed on surfaces is more active than pure DDT i.e. a smaller dose per unit area kills Cimex. But the lasting power of the benzene hexachloride is less (BARNES, MS).

It is clear that much remains to be done on the persistence of DDT

applied to surfaces The type of surface will greatly affect the persistence of the insecticide deposit from a solution suspension or emulsion, rate of evaporation of solvent and many other factors may all affect the matter The suggestion has been put forward that the DDT might be incorporated either in a distemper or in an oil paint, and some preliminary work has been done which appears encouraging, but the dose of DDT per square foot is not stated (CAMPBELL and WEST, 1944b) It would seem evident that this method must be somewhat wasteful of DDT, because so much of the insecticide must be buried in the thickness of paint, but convenience of application might render this worth while.

Practical extermination of bugs with DDT has already been carried out in jails, barracks and ships in many parts of the world The exact dose to be put down is not capable of precise definition, for it seems certain that over wide limits the more one puts down the longer it will last. It seems that about 100 mg per square foot is likely to be satisfactory and to kill any bugs which may be brought in for some 3 months. Some such dosage may be attained if one uses 5 per cent. of DDT in kerosene (i.e a nearly saturated solution at temperatures prevailing in Britain, or 7 ounces per imperial gallon) and sprays with a coarse nozzle held close to the wall, putting on enough spray to make the wall look wet, without the liquid running off One quart of spray should cover 300 square feet The operator's knowledge of the haunts and habits of the bug might be a large factor in success

DDT dusts (5 or 10 per cent.) have been used successfully Under the circumstances which generally prevail in houses a dust is more likely to be removed than a spray deposit, and therefore less likely to give a satisfactory lasting effect.

There is at the moment no information on the effect of DDT on the tropical bed bug (C rotundatus) All the work has been done on C lectularius

6 OTHER DOMESTIC INSECTS.

It is certain that a film of about 100 mg per square foot would kill many other types of insects which occur in houses. Its importance in control of house flies and house haunting mosquitoes is emphasized elsewhere

Cockroaches appear to be somewhat resistant to DTT at least in the form of 5 or 10 per cent. dust. It seems that they are also rather resistant to films There are several reports indicating a great reduction, but not complete extermination, of cockroaches of several species BUSVINE, for instance, has recently sprayed a bakery infested with Blatta, putting down a film estimated at 100 to 150 mg per square foot. Those insects which were hit during the spraying died but there were many live ones running on the film a week later

Pharaoh s ant (Monomorium pharaonis) is a most difficult insect to eradicate We have no precise data on the effect on it of DDT but a field test in which the DDT was applied as a 1 per cent solution in kerosene was disappointing

A further trial in which the concentration was put up to 5 per cent and the walls sprayed to leave about 100 mgm of DDT per square foot achieved a very great reduction in infestation (J. R. Busvine, unpublished).

Against fleas there seems to be little doubt that a 5 per cent DDT powder is very effective—the only published information is a brief note by Lindquist, Madden and Knipling (1944). It would doubtless be possible to "proof" a dog or other animal by wetting its fur with a DDT emulsion.

It is certainly possible to use DDT to make fabrics, cartons for food and other materials, proof against many types of insect for prolonged periods. Dosage and period of protection remain to be worked out though one or two preliminary notes have been published.

7. ACARINA (MITES AND TICKS).

There is unpublished information from McCulloch working on DDT against Trombiculid larvae, with Australian Forces in New Guinea. Freshly impregnated garments protect man from attack. But if garments impregnated with DDT are worn and washed, some larval mites succeed in attaching themselves and bite after the third wash. Individual larvae were allowed to run on cloth freshly impregnated with 1.5 w/w DDT and were paralysed in about 30 minutes. After the cloth had been twice washed in cold water the time was 120 minutes. One presumes, therefore that this method of protecting men against the vector of scrub typhus is not of much value.

Experiments with insecticidal dusts sprinkled over herbage much infested with two species of Trombiculid larvae are reported from Georgia and South Carolina, U.S.A. It seems that 30 lb per acre of 2 per cent DDT in mineral powder was effective in reducing the mite larvae—in one experiment they were reduced to 2 per cent or less of previous number for 7 days—in a second experiment (125 lb of 1 per cent DDT) the reduction was only to 19 per cent. Elemental sulphur and dinitro-o-cresol at 60 lb per acre were about equally effective (Smith and Gouck, 1944).

As to Ornithodorus very little is on record. Robinson (1944) tested a number of dusts, putting O. moubata to crawl on the material for 24 hours in an open dish at 28° C. Undiluted commercial DDT was not at all effective, four out of ten ticks being dead 6 days later. Ruda and Smith (1944) working in Texas applied DDT in a non-drying adhesive to the inside of the ear of 113 cattle. The material gave a satisfactory kill of ticks (O. megnini) which were present at the time of application and afforded some protection from re-infestation. The same treatment was more effective against Amblyomma maculatum, killing ticks which had attached when the material was put on, and giving good protection for 3 weeks.

Experiments on the control of Rhipicephalus sanguineus on dogs are described by Gouck and Smith (1944). They used a variety of emulsions containing 5 per cent DDT and report complete success against larvae, nymphs and

adults of this tick whether attached or walking on the dogs. Some are killed and drop off within an hour others not for several days. A 10 per cent. DDT dust has also been used with great success.

SUMMARY

Dichlor diphenyl trichlorethane ('DDT') is a remarkably specific substance. It has very great insecticidal powers, indeed against many insects it is effective in smaller doses than any other synthetic insecticide it is also very general, being poisonous to all or nearly all insects, or indeed Arthropods. It combines this high and general insecticidal power with low toxicity to mammals. If applied to the skin or swallowed in the presence of a solvent DDT can indeed be absorbed large doses can cause pathological changes especially in the liver, and produce symptoms of which tremor is generally the earliest. Using appropriate solvents and large doses there is no difficulty in showing that DDT is toxic to experimental mammals. There is, however no evidence that the substance has proved harmful to those who manufacture it, or use concentrates of it, or apply it in the field. After 2 years of very wide experience, I feel that we may say that, used as an insecticide DDT is harmless.

It is certain that DDT is a contact poison the solid material penetrating the surface of insects a common port of entry is the tarsus. An early symptom is muscular incoordination. Whether DDT is also a stomach poison is not known. It has no fumigant effect. On most insects the effect is slow so that there is no immediate knock-down. It is not ovicidal, and not repellent. DDT is not fungicidal.

The applications of DDT against particular insects are manifold. In relation to hygiene the most remarkable are —

1 Cotton or woollen garments may be impregnated and retain the power of killing lice after being worn some weeks and washed several times

2. A film of DDT may be deposited on a wall and will kill flies or mosquitoes for many weeks.

3 DDT in a mineral oil kills mosquito larvae, the volume of oil required is a tenth or twentieth of what one would require if the oil were used without DDT

One should insist that it is inadequate to think of DDT as a substitute for some other material or merely as a new and excellent insecticide. In several ways it opens up entirely new possibilities. If we are to exploit it fully we must think freshly, and carry out research in addition to technological development.

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Discussion

The President (Sir Harold Scott) DDT is a subject of interest to many branches of the profession, and many here will wish to speak on the subject and ask questions. I have one question to ask Professor Buxton there is no mention in the paper or in any paper I have seen dealing with the effect of DDT of any action it may have on ticks I am thinking of the tick-borne diseases which are common in the tropics, particularly tick-borne relapsing fever and wondering whether DDT might not be effective in that connection ?

Dr George Macdonald Professor Buxton has done a valuable service by making such a comprehensive review of DDT for which I would like to thank and congratulate him.

I repeat his hope that the field workers will soon have an opportunity of putting their work together and publishing it under their own names. My own experience is so bound up with that of several colleagues that I cannot separate it from theirs, and I trust they will forgive my referring to their work.

In the course of 6 months experience in the field use of DDT in which several tons have been used, generally in the form of 5 per cent. solution, no case of systemic poisoning has been seen, though two cases of heat exhaustion attributable to the use of excessive waterproof clothing have occurred. I have also seen one case of dermatitis, following the careless use of a 5 per cent. solution in kerosene for a period of 2 days. The eruption was papular vesicular and pustular on the extensor aspect of the arms and legs, the back of the hands and feet, the webs of the fingers and the outer aspect of the right thigh. There was no evidence to differentiate it from a kerosene rash.

The main use of DDT has been as a residual insecticide, and of necessity practical work has been run alongside or even ahead of experimental work. Even extremely low doses of the order of 50 mg per square metre (10 8 square feet) have some effect though this is very temporary With doses of 200 mg per square metre or more, the treated surface remains lethal to the *maculipennis* group of anophelines for a period of 10 weeks Higher doses give a slightly prolonged effect which is not in direct proportion to the amount applied. In practice I consider that a dose of 500 mg per square metre should be aimed at and it should be considered to be effective for 8 weeks.

DDT has been applied for this effect in the form of kerosene solutions, emulsions and as a dry dust. The results appear to be much the same with

oil solutions and emulsions but are less satisfactory with the dust. As a preliminary precaution, the oil used as a solvent should be tested to ensure that it is not repellent, a practical possibility which has been at one time a serious source of trouble.

Following the application of these doses, in solution or emulsion, there is an immediate reduction of the anopheline population, always to 2 per cent or less of its previous density and the density in control rooms. When dust is used the reduction is to about 10 per cent. of the previous figure. The anophelines remain at this negligible figure for 8 to 10 weeks when there is a slow increase in their numbers, though unfortunately I have no records prolonged enough to show the complete return to normal figures. There is clear evidence that this is not due to repellent effect a point of extreme importance and that it is due to actual killing of mosquitoes I have seen, in some treated houses near to a very extensive breeding place, a thick mat of dead anophelines on the floor. A typical result, observed by Major M. Yom, when farm living rooms and rabbit hutches were left untreated and stables and cowsheds were treated with 560 mg DDT in oil per square metre on 8th August is shown below.

Number of anophelines observed.

	August					September				October		
	7	11	13	18	24	1	10	15	25	10	21	31
Bedrooms	2	0	0	0	1	0	3	3	3	0	0	0
Rabbit hutches	0	100	25	50	34	70	40	15	50	40	20	25
Animal sheds	460	0	0	0	0	0	0	0	0	0	0	4

One might legitimately hope that the destruction of anophelines would be so great and the passage of living specimens from room to room so frequent that there would be some reduction in the number of anophelines in neighbouring untreated rooms but despite careful observation no evidence that this is the case has been found it is equally important that no repellent effect is exerted and followed by an increase in neighbouring places.

The solution has been applied with a variety of spraying machines though the ideal has not yet been found. The power driven distributor the bomb referred to by Professor Buxton or any other form of apparatus giving an aerosol, is certainly not the most suitable there is a great tendency for the spray to be deflected from the wall, and it is extremely difficult to wet the higher parts of a high room. A machine is needed giving an even cone of fine droplets above the aerosol size, with a long reach, readily portable, and ample to use by semi-skilled or non-skilled labour. The most satisfactory one I know is the Four Oaks Ross machine with a 1/32-inch humidifying nozzle, which at normal speed of operation gives about 50 c.c. to the square metre. It is difficult to adjust the delivery of fluid below this and therefore it is desirable

to adjust the strength of solution to 2 per cent. or less to get the correct dose of DDT A more satisfactory apparatus could be made by the development of the stirrup pump to suit the particular needs of this work.

As Professor BUXTON has said, it is necessary to select the most suitable shelters for treatment and in Italy it was found that the population of the *maculipennis* group was about thirty times as high in animal sheds as in houses even when they actually adjoined. This agrees with previous evidence and does not mean that the anophelines are feeding exclusively on animals Work on animal sheds has therefore been given priority over work in other places.

Application is definitely slower than application of pyrethrin insecticides with power apparatus. In rural areas a gang of ten men using one vehicle have been able to do about twenty separate farm houses in a normal day's work, spraying 3,500 square feet in each In towns the normal task is bigger as the travelling is reduced.

The data I have given refer to rough plaster walls and to the *maculipennis* group of mosquitoes. I have no data on the correct dose for thatch and such like surfaces or for other mosquitoes Further information is also needed on the effect on the population in untreated rooms in the neighbourhood where it would seem logical to assume that there must be some difference in the age distribution of the mosquitoes.

Apart from early experimental trials I have no experience of my own of DDT dust as a larvicide. The early trials did not suggest that in small doses it had any permanent effect, and for its immediate effect it seemed to have no advantages over the much more readily obtainable paris green or copper cyanide

When applied in oil solution 10 mg per square metre in an unbroken film destroys all mosquito larvae, but in my experience does not produce any permanent or semi permanent effect such as that described by Professor BUXTON and other workers This difference may be due to the different types of surfaces treated, the DDT being blown away in my examples, or to differences in the oil vehicle affecting the stability of the film. With doses of this character applied as a 5 per cent. solution the resultant film is of the order of ½ micron thick films of this thickness are known to be unstable particularly when oleic acid is used as a spread aider and more work is needed on the character of these films before we can correctly compare dosages of this type

The immediate destruction of larvae is complete and, when large areas are treated from the air dramatic on one large swamp several square kilometres in area which before treatment had yielded several larvae per dip it was only possible to find half a dozen larvae after elaborate search on the next day With extensive treatment the associated decrease in adults in neighbouring houses is equally dramatic. Major CRAUFORD BENSON noted a decrease of the gross count in six control rooms from 2,338 to 31 10 days after treatment, but whether entirely as a result of larval destruction or partly as a result

of destruction of egg laying adults I do not know It is interesting to note that when paris green was substituted for DDT as a larvicide in this case, the adults in neighbouring houses increased by 10 per cent. of their previous number

The development of DDT is undoubtedly revolutionizing military malaria control to what extent will it justify its advance reputation as a panacea when applied to the needs of civilian rural populations?

The work of RUSSELL and his co-workers has shown the extreme value of adult control in rural communities. DDT properly applied is a far more effective insecticide than the one they used and will bring malaria control within the reach of populations which could not or did not previously afford it. Considerable preliminary entomological enquiry will be necessary and the correct dosage for thatch and other common types of wall will have to be worked out.

As a larvicide it will produce a less dramatic improvement and unless care is taken to ensure that it is properly developed it will meet the fate of paris green which has quite wrongly become discredited in many large areas. Application from the ground reduces the amount of oil needed from 20 gallons to ½ gallon per acre it might be said that the use of paris green similarly reduces the weight and cost of larvicide used. The labour of application remains much the same. Supervision is more difficult and consequently more highly skilled labour must be employed. There is physical difficulty in the application of such small quantities evenly over large water areas, and the types of apparatus now in use are unsuitable. If it is used as a mere substitute for oil little reduction in costs or increase in efficiency will result, but they could be achieved if it was no longer necessary to ensure application to every part of the breeding area or if a prolonged larvicidal effect could be secured. To get its full value we need work on the development of a solution with high spreading qualities, producing a permanent film, and penetrating into vegetation, which might be applied at one part only of the breeding place with certainty of wide spread. An alternative is a continuous production of surface films from a solid vehicle on to which the oil solution is absorbed. BARBER suggested such a means, and I have seen a prolonged effect in small water areas following the introduction of balls made of a plaster of paris and sawdust mixture incorporating the larvicide

Dr J R Busvine We are very fortunate in having, at last, an authoritative account of this new insecticide. One of the things which must have struck us, I think, is the very great deal of technological work that has to be interposed between the discovery that a new substance is highly insecticidal, and the actual use of that material in the field. During this war we have had two or three major insect borne diseases to cope with, and have had to protect troops against these. Among the insects concerned were the typhus-bearing louse and the malaria and yellow fever-carrying mosquitoes

and a great deal of the work that has been done has been on these particular insects. We have heard some interesting dramatic experiences about the control of the mosquito and I would like to add a few notes about the control of the louse. I do not think I am quite so cautious as Professor Buxton in ascribing the conquest of the louse to DDT, and I would like to quote Professor Buxton himself in relation to it. Quite early in the war he saw that there was the likelihood of another louse outbreak and the dreadful epidemics that are associated with it, such as there was at the time of the last war. His opinion was that the reason why the louse controlling measures of the last war had failed was because of the lack of prevention of reinfestation, so that people who were de loused speedily became reinfested. He set to work, and early in the war I joined him and we looked for materials that could be applied to clothing and retain for as long as possible their louse killing effect. We tested some of the better known insecticides such as pyrethrum, and some of the organic chemicals such as thiocyanates. We developed a method of proofing louse-infected men against lice for 3 or 4 weeks. Pyrethrum was not entirely satisfactory partly because of the the shortage of material and partly because it tended to oxidise or break down chemically when exposed to the dirt and sweat of the human body when unable to wash. Thiocyanates proved better and were our final choice because they gave a louse protection for 3 or 4 weeks but they were liable under certain conditions to cause irritation to parts of the human body. At the time we thought them better than a typhus epidemic. When DDT came to our notice we found that it was very toxic to the lice, very lasting in its effects and innocuous to man. It seemed to be the ideal insecticide for this purpose whether applied as a dust or impregnant and therefore it is not surprising to me that the Naples epidemic should have been quelled by it because if you dust a man's underwear with this material it remains toxic to lice for about 3 weeks. It is true that the simplicity of the treatment and the speed with which it can be done contributed a great deal to its success. In the demonstration you can see the primitive little "gun" that is used for dusting up clothes without undressing people. But I think it is also a fact that the very toxicity of the material to lice enabled this simple measure to be effective. From the experiments in which we have treated infested vagrants we know how very readily people can become reinfested when living in an infested environment, and unless a treatment is fairly lasting it is not able to check lousiness. The impregnation of garments is even more valuable for military personnel because they can be issued with impregnated shirts in large numbers and the experience of our armies in the field in this war is very different indeed from that of the last war. Many of the old soldiers of the last war to whom I have spoken in London air raid shelters and elsewhere, assure me that they were chronically lousy and it is clear that about nineteen out of twenty were infested. We have to thank DDT for the improvement that exists now. A great deal of our attention has been devoted to the

louse and the mosquito and before we can really use the DDT for the pests that are going to worry us in peace-time, still more work must be done on these other pests. I am afraid it is not quite clear why this is so, but there are so many different ways in which DDT can be used—as a powder a film or in various solvents emulsions and dispersions—that to make the best use of it, it is important to get it into the right phase.

I would like to mention the two pests to which I think attention should be given at present which otherwise cause trouble in peace-time   they are the ordinary house-fly and the bed bug   In this country I have seen many house-fly infestations "—large numbers of house-flies in habitations— though in general they were not associated with epidemics of disease. I think that is simply a reflection on our sanitary system and good drainage. Flies undoubtedly contaminate food, but evidently they do not as a rule have access to dangerous infected matter   There is one exception, and that is the hospital. Many hospitals have been far too heavily infested with flies in my opinion, and very often they have been accompanied by some small outbreaks of disease. One of the urgent problems is to adapt DDT to treating the walls of hospitals and subsequently other buildings. Perhaps in the tropics the house-fly is well known to be serious, and there is no need to labour the point here. With regard to the bed bug   we all know of the bed bug as a serious problem in the slums before the war   which we were trying to defeat by slum clearance and the use of cyanide   But there again was the problem of reinfestation   people were put into clean, newly built premises and infested them by buying second-hand furniture   After the war we shall have to do a lot of housing not only by building but with prefabricated houses.   Where we use these prefabricated houses I think there is likely to be a particular danger of bug infestation, because all these houses made in sections are very liable to have cracks (quite naturally) between the sections.   It is very difficult to seal these adequately because of the normal expansion and contraction by heat   particularly if metal is used and this, together with the use of hollow wall spaces for insulation, makes some of these prefabricated houses that I have seen ideal breeding places for bugs. I think that the first considerations of our home peace-time programmes for DDT are the attacks on the house-fly and the bed-bug

Dr Kenneth Mellanby said that Professor Buxton had shown that the discovery of the properties of DDT was probably the greatest advance in insect control which had ever been made   But DDT was clearly no panacea, which could be broadcast indiscriminately to kill off all noxious pests. A greatly increased amount of field research was necessary whenever DDT was used.   Fortunately   mosquitoes and muscid flies seemed particularly susceptible to this substance but all other arthropods were affected to a lesser or greater extent.   Much work should be done on its effect, in the field, on all manner of apparently unimportant insects and other forms of life, to ensure that there

was not a serious upset of the balance of nature with subsequent disastrous effects.

Excellent as is DDT it is *not* the perfect insecticide and further work is needed to find even more effective substances. The Arachnida, for instance are apparently much less susceptible than the insects Thus DDT is of little value in the control of mite borne typhus

Against human scabies DDT is surprisingly inefficient. A saturated solution in oil, or an emulsion in water applied to the skin, kills less than 50 per cent. of the *Sarcoptes* in 24 hours whereas benzyl benzoate emulsion or sulphur ointment will kill well over 99 per cent DDT is certainly not to be recommended for the treatment of scabies

Brigadier J A Sinton We have heard a most instructive account of the history of DDT and of the experimental work from Professor BUXTON and Dr GEORGE MACDONALD has told us of the results of his work in the field in Italy I think a few remarks on the important trials being carried out in the Far East under the auspices of the Army would be of interest

Colonel SCHARFF has been conducting a very extensive series of trials of DDT against the mosquito not only in India but also on the Assam Burma Frontier This insecticide has been used successfully from the air and also from the ground I do not propose to discuss the large subject of distribution from aircraft, the results of which have been in most instances better even than we anticipated.

The effects of residual spraying against adult mosquitoes have been so good that it seems possible that this may prove to be the best method of using DDT for routine malaria control in civil populations I should like to quote some extracts from the latest report by Colonel SCHARFF about the results of such spraying Almost 100 per cent. reduction of anopheline population inside village huts was maintained for 1 month after DDT spraying and a substantial reduction for at least a further month. He considers that, theoretically thorough spraying once every 3 months should suffice but in practice under service conditions to derive the maximum comfort and protection he recommends re spraying monthly

These results were obtained by the application of a 5 per cent. DDT solution in kerosene at the rate of 1 quart per 1 000 square feet (or about 50 mg per square foot) On the other hand, a team working in East Africa under the direction of Colonel BAGSTER WILSON report that they needed doses as high as 200 mg per square foot to get the maximum effects in tents but that 50 mg gave good effects for as much as 3 months after application.

These different results appear to indicate that the optimum dosage will vary with the type of surface to which the residual treatment is applied, and probably also with the species of mosquito implicated. This requires further investigation

The work which has been carried out by Army workers has been most promising and the results extraordinarily good. Colonel SCHARFF concludes that " experience has shown that DDT is more potent than a theoretical analysis of its killing powers would permit us to assume. It is much too early yet to assume that we have reached the millennium in so far as mosquito destruction is concerned. We have still very much to learn about both the potentialities of this marvellous insecticide and its limitations and also about the best methods for its practical application under the very varied conditions encountered in the field.

Dr C M Wenyon   Has anything been done in connection with the domestic clothes moth ?

Professor Buxton (in reply)   With regard to the question about DDT and ticks little is known   It is summarized in the text. Broadly speaking, the evidence is that some ticks require high doses, by the methods of application which have been used up to now

As to the clothes moth, I have no detailed information, but I think I am right in saying that impregnated garments are moth proof and remain so for a very long time

Professor BUXTON also referred briefly to certain demonstrations, of pure and technical DDT and of impregnated " louse proof " Army shirts.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

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ANNOUNCEMENTS

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

A SPECIAL MEETING OF THE SOCIETY

to commemorate the

100th Anniversary of the Birth of

SIR PATRICK MANSON K.C.M.G M.D F.R.C.P F.R.S

held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 14th December, 1944 at 8 p.m

THE PRESIDENT

SIR HAROLD SCOTT K.C.M.G F.R.C.P F.R.S.E.

in the Chair

ADDRESS

THE MANSON SAGA

3RD OCTOBER, 1844—9TH APRIL, 1922.

BY

SIR PHILIP MANSON BAHR C.M.G D.S.O M.D., F.R.C.P

We are celebrating the centenary of PATRICK MANSON who was born on 3rd October 1844 This may seem somewhat strange to many who are still here, and who remembered him in his prime as well as to others of the younger generation, to whom the Manson saga if I may use the expression is still a living inspiration. So we are assembled here today to praise MANSON but not to bury him, for this can never be whilst this meeting is held in this shrine so appropriately dedicated to his name

Those who have particularly studied the history of tropical medicine are familiar with the many discoveries which have rendered his name immortal. They have been recorded already many times in books and memoirs and no good purpose would now be served by enumerating them in detail once more. He stands high in the realm of parasitology where several species and genera hand down the name of MANSON to posterity so that, in addition to his title of the Father of Modern Tropical Medicine, he may well be considered the Godfather of Medical Helminthology and Medical Entomology and even of Mycology for sometimes the fact is too easily overlooked that one of his earliest original papers referred to the discovery of the fungi of tinea imbricata in 1878.*

Not only can one justly claim his pre-eminence as the result of his innate ability in these widely separated fields, but we must remember that, to the ordinary man he embodied the personification of a great and well-beloved physician and was best remembered as a sane, sympathetic and eminently practical doctor by his patients in Amoy Hongkong or London—wherever his footsteps led him.

MANSON achieved much besides making scientific discoveries. Did he not introduce vaccination into China and render it a popular measure amongst the illiterate masses, who saw much more clearly than many of their so-called more enlightened fellows that it spelt safety from the ever prevalent virulent smallpox ? The original and rather brutal glass-cutter like vaccinator which he used at this time is now preserved in the museum of the London School of Hygiene and Tropical Medicine Was he not a skilled gynaecologist, held in high esteem by both European and Chinese women in their hour of trial, and did he not bring many into the world who have since risen to high estate ? Was not his knowledge of ophthalmology far above the average and did he not undertake many delicate operations on the eye which today are reserved for specially trained surgeons and lastly was he not a most competent surgeon as his lists of operations performed yearly from 1871 onwards and his early reports from Amoy abundantly show ?

It is true that he once described himself as an indifferent surgeon, but a good carpenter but this rather derogatory statement omits reference to many novel surgical procedures which he introduced. These included the methods he devised for his operation for elephantiasis scroti of which tumours he confessed to having removed over a ton in the year 1874 Nor should one fail to mention his extremely ingenious trocar and cannula for the drainage of liver abscess designed to avoid a major operation which at that time was apt to be followed by a high mortality MANSON's trocar has survived to the present day and, in a modernized form, now figures in the armamentarium of

MANSON P (1878) Notes on Tinea Imbricata, an undescribed species of Body Ringworm. *China Imperial Maritime Customs Reports.* Special Series No. 2. 18th issue, pages 1 to 11

the genito-urinary surgeon as a method of draining a distended bladder (suprapubic cystotomy)

Of the most distressing surgical emergencies the most common were genito-urinary complications caused by stone and he early became involved in surgical procedures for their relief and acquired considerable skill in perineal lithotomy but he was foiled and baffled by the suspicious attitude of even the better educated Chinese to Western medicine, especially where cutting operations were concerned. He realized that mistrust of operative surgery must be gradually overcome, but that most of it arose from ignorance of what happened to their friends and relations in the dark interior of hospital wards They had an idea that entering at the front door the patient passed out into a coffin at the back so MANSON conceived the idea of making his operations a public demonstration by constructing a large window in the operating room, through which the public could observe what was being done. They could see for themselves the safe and successful removal of a stone from the bladder and the patient transferred still alive to his bed and they could see him the next day sitting up serene and happy Soon after having induced their more lowly brethren to submit to operation as an experimental venture the higher-class Chinese, the mandarins came to consult him and eventually also permitted him to operate and so in this manner he finally won complete confidence of the Chinese population

There are many tales of his originality and ingenuity as a practitioner which are worth retelling There was one, I remember of a patient whose life he dramatically saved. This concerned a man of mature years who had suffered from multiple renal calculi Some years before the right kidney had been removed for pyonephrosis and now the remaining left was in a similar state. An operation for drainage of the pelvis had been successfully undertaken and a drainage tube was in position. Suddenly in the middle of the night with onset of great pain complete anuria set in and MANSON was summoned. With that rapid insight which so distinguished him, he realized that a calculus had become detached and wedged in the neck of the pelvis so taking a metal catheter he inserted it into the wound Feeling it impinge on a hard, solid object, he gave a sudden push and, with a gasp of relief the patient recommenced to pass urine accompanied by the stone I can still recall, when he told this story the expression on his face and the look of triumph as he described how the obstructing calculus fell flop to the bottom of a domestic article held ready for its reception.

Then there is a story from his Formosa days An American naturalist, who had been exploring in the interior had been suffering from uncontrollable epistaxis since he quitted the jungle. When he arrived he presented a woebegone figure with blood stained handkerchief and equally blood stained face Taking a nasal speculum, MANSON observed on the upper nares the forbidding tail of a jungle leech by drenching it with a syringe-full of hot

salt water it soon released its hold and fell into the awaiting kidney dish. The American was so flabbergasted at this apparition that with an exclamation of My God, he dropped the dish and fled never to be seen again.

During his 20 years of consulting practice in London he earned the respect and confidence of numerous surgeons for his skill in locating liver abscesses. In this he was specially associated with Sir Rickman Godlee, the biographer of Lister. There was one occasion when a distinguished patient, whose condition suggested liver abscess, lay anaesthetized on the operating table, whilst three successive surgeons endeavoured by means of the aspirating needle to locate it. Eleven punctures had already been made without success, when Godlee turned to Manson and said. What about the twelfth for you." With his trembling hand (for many years he was afflicted with a gouty tremor of his right arm) he grasped the syringe and, directing it in an upward and inward direction towards the right dome of the diaphragm struck the abscess, thus enabling the operation to proceed successfully.

With all this practical application to his profession it is amazing that he found time to dabble in so many abstruse fields. We know that he took a great interest in leprosy but was ignorant of its cause, while the true import of Hansen's discovery had not yet reached him, and so we find him making experiments to discover the germ and if we can trust the accuracy of some drawings in his diary he appears to have succeeded in demonstrating leprosy bacilli though he did not stain them (1874) but what is still more interesting and suggestive is the record of his attempt to cultivate these bodies, by inserting capillary tubes, filled with leper juice, into hen's eggs and using the mother bird as a natural incubator. In this manner he anticipated by some 50 years the modern method of cultivating ultramicroscopic viruses. When one reflects that Pasteur's germ theory of disease was communicated to the French Academy only in 1878, we can almost claim that Manson anticipated both him and Koch in the artificial cultivation of bacteria.

In addition to all his qualities Manson excelled as an organizer as witnessed by his success as first Dean of the Hongkong Medical School which finally blossomed out into a University and even by his institution of the Hongkong Dairy designed to provide clean cows' milk for the children of the military garrison and other Europeans in the island, and staffed by stout yeoman farmers from his native Aberdeenshire. This institution eventually proved to be one of the greatest benefits ever conferred on that colony. Finally the seal was set on his fame by the foundation and conduct of the London School of Tropical Medicine, as will be related by his pupil friend and colleague, Dr G. Carmichael Low.

One of the greatest drawbacks to medical research in China was the difficulty of examining the dead to which the Chinese were fanatically opposed. Manson's zeal was from the first directed to the discovery of the adult stage of the embryo filaria he had studied in the blood. At one time he bargained

for the body of a patient with lymph scrotum who was nigh unto death and had even paid the prospective widow a retaining fee of 100 dollars When the day arrived he, with his brother DAVID (who had joined him in Amoy) entered the death chamber No sooner had he started to dissect the scrotum than there arose in the courtyard a terrible hubbub and the cry of Foreign devils rent the air Snatching those portions of tissue they had removed and cramming them into their pockets they holted out of the backdoor in time to escape from the howling mob but he had his reward by finding the hair-like filaria in the lymphoid tissue, but alas he was too late for he was duly informed by COBBOLD with whom he had corresponded that the worm had already been found and described by BANCROFT in Brisbane 2 years earlier and also by LEWIS in Calcutta. As is well known BANCROFT received priority and had the honour of providing the specific name for this parasite, *Filaria bancrofti* So elated was MANSON at the elucidation of his long-cherished hypothesis that my wife, who was born about this time, narrowly escaped being christened Filaria, which would have provided her with an original but startling Christian name

Another equally risky exploit led to the discovery of *Sparganum mansoni* On this occasion he entered a Chinese cemetery at night and by the dim light of a lantern proceeded to dissect the remains of a dead Chinese he had been observing Amidst the ghostly surroundings he discovered the tape-like structures long known as *Ligula* or *Sparganum mansoni* now more correctly classified as the plerocercoid stage of the tapeworm, *Diphyllobothrium mansoni*

Being deprived of human material MANSON had recourse to dissection of dogs and birds and endeavoured to check the ways of *Wuchereria (Filaria) bancrofti* by comparative observation on lower animals. In the native dogs he found the embryos of the heart worm *Dirofilaria immitis* he described the parent forms accurately and made beautiful and accurate drawings of their anatomy which have been preserved in his Diary He noted that the embryos maintained a tendency to nocturnal periodicity and congregated in the pulmonary arterioles during the daytime

In the familiar Chinese white necked crow he discovered a new species —*Filaria corvi torquati*—of which the parent forms live in the pulmonary artery and in the local magpie still another species came to light. This was *Filaria picaemediae* the adults of which were found coupled together in minute tubercles under the endocardium of the semilunar valves But further research was prevented by native superstition and as he wrote Being denied the opportunity of necropsies in man I had to make use of dogs cats and birds and had found that the blood of the magpie contained at least two species of filaria, so I shot as many of these birds as possible but the Chinese told me that I must stop my work in this direction because the magpie is a sacred bird in China, tradition holding that, many centuries ago, the spirit of a defunct Emperor had entered one of these birds. Therefore it was possible that either I or my friends might shoot this particular fowl

In the horse he found the eye-worm, *Filaria papillosa*, and was intrigued by a special boring apparatus, shaped like an old-fashioned trephine, around the mouth from this he inferred that the eye was not the final resting place of this species again in the eye of the domestic fowl he discovered an entirely new parasite, *Oxyspirura mansoni*.

He appeared to be interested in all diseases of domestic animals and at one time we find him actively engaged in investigating an outbreak of trichiniasis in pigs.

An aroma of romance entered into many of his major discoveries, although he himself did not realize at the time that he was engaged upon anything extraordinary or important. Some may have read the story of his demonstration of the nocturnal periodicity of *Wuchereria bancrofti* which eventually led to the discovery of its life history He drew great conclusions from simple observations and based his hypotheses upon them—observations which the ordinary man would have passed over without comment.

Early in 1876 he engaged two Chinese students or dressers to assist him in his hospital work in Amoy and had trained them to carry out a systematic survey of the blood of Chinamen in the wards. They brought him the thick-drop specimens of blood which they had prepared for examination by Manson with his primitive Nachet fils simple lens microscope. (It is interesting to record that, although no model of this instrument is now available, the author saw an identical one at Rochester U.S.A., where it is preserved in the Museum as a relic of the original William Mayo of the Mayo Clinic.) One of these students was on duty during the night hours as he had a sick mother at home and, with that praiseworthy filial domesticity of the Chinese, was wont to dance attendance on her during the day whilst the second, having no such ties, worked in the normal manner To Manson's great satisfaction it was the night boy who invariably scored the best bag and presented specimens of blood with the greatest number of microfilariae This singular but patent, fact struck Manson as portentous and he divined that the only tenable explanation lay in the fact that these parasites congregated in the peripheral blood during the hours of darkness. As he said at the time, "It is the discrepancy which teaches, if you would learn. Where the microfilariae went during the daytime was a question he waited another 20 years to solve, when a West Indian patient with *W bancrofti* infection conveniently decided to commit suicide at 8.30 in the morning and Manson was able to obtain sections of his lungs and to demonstrate large numbers of microfilariae in the capillaries (as is well illustrated in successive editions of *Tropical Diseases*). These early observations led to his enunciation of the law of periodicity and the construction of his well known chart, in the execution of which (all in his own hand) he made systematic 4-hourly blood examinations of his gardener —Hin-To—over a period of 6 weeks. This chart, which was correlated to the temperature, pulse, respiratory rate, atmospheric and barometric readings,

must still be regarded as a model of its kind in execution as well as of industry. The regularity of the nocturnal incursions of microfilariae into the blood, combined with his beautifully executed experiments on the discarding by the embryos of their sheaths, when exposed to the air led to his mosquito experiments in his specially devised cage, again using the patient and uncomplaining Hui-To as his victim. The outcome of the first part of this work was communicated by Cobbold to the Linnean Society of London where it was received by much banter and chaff and with a number of questions as to whether the filariae carried watches so as to ascertain their exact bedtime. The second part constituted the corner stone of tropical medicine and was crystallized in his historic paper On the Development of the *Filaria Sanguinis Hominis* and on the Mosquito considered as a Nurse. [*]

And so we have his humble statement in his own words that he had stumbled on an important fact with a distinct bearing on human pathology" and, speaking about his discovery at a later date he used these words I followed it up with the meagre appliances at my disposal [he used a fine pen nib for dissection of his mosquitoes] and, after many months of work, often following up false scents I ultimately succeeded in tracing the filaria through the stomach wall into the abdominal cavity and then into the thoracic muscles of the mosquito. I ascertained that during this passage the little parasite increased enormously in size. It developed a mouth an alimentary canal and other organs. Manifestly it was on the road to a new human host

Spencer Cobbold accepted Manson's work, but other authorities were by no means enthusiastic. Nettled by the polite doubts of Lewis Manson in 1883 repeated and amplified his observations of 1877 and communicated the results with historic illustrations again through Cobbold to the Linnean Society[†]

But this does not by any means close the chapter of this decisive stage in the history of tropical medicine. We know that he wished that his great discovery should be verified at first hand by the best authorities in Europe of whom Spencer Cobbold was chosen as the referee. There exists in his Diary in Lady Manson's handwriting (as his amanuensis his wife played a prominent part at this time) a letter to Cobbold from Amoy dated 20th June 1879. I will forward you by this mail filaria impregnated mosquitoes. They are preserved in glycerine and were fed on the blood of the man whose case I append. The letter ends with this pregnant phrase. Men, like myself in general practice, are but poor and very slow investigators crippled as we are with the necessity of making our daily bread. But the does end here. What became of these specimens? We now their fate. We know that one bottle apparently its way into the house of Stephen Mackenzie,[‡] where

[*] Manson P. (1878.) *Trans Linn Soc Lond.*, 14 No
[†](1884.) *Trans Linn. Soc. Lond. Zool.*, 11, pt. 10
[‡] Letter to Ross, 23rd December 1893

in 1896 when he was preparing his Goulstonian Lectures on the *Life-history of the Malarial Germ outside the Human Body*.[a] In the giveratne the mosquito was well preserved and in sections which were made of its thorax there were the larval filariae in the sausage stage "too beautiful to behold," and this specimen figured not only in that lecture but in the early editions of *Tropical Diseases*. COBBOLD was enthusiastic, read and published a paper claiming to have dissected some of the preserved insects which MANSON had sent him and to have verified his discovery. But listen to the sequel. About the year 1935 the Curator of the Royal College of Surgeons notified Professor LEIPER that some helminthic material had been discovered in the precincts of the College and would he like to investigate it? There he found a satin-wood box, duly sealed and bearing the *Amoy* postmark, and addressed to COBBOLD in LADY MANSON's handwriting. On opening it, six bottles leather capped and sealed with paraffin, secured by fine twine, were discovered exactly where MANSON had placed them in 1879. Each bottle was labelled and dated in his own spider like hand and in each there can still be seen beautifully preserved mosquitoes (*Culex pipens*). So it is incontestable that these were the original specimens despatched by MANSON as already related.

MANSON's share in the discovery of the lung fluke (*Paragonimus*) provides a good example of homely observation. The story begins in 1878 with a Portuguese patient from Formosa who was under his care in Amoy suffering from a thoracic aneurysm. The next year this man returned home to Formosa, where he soon died. The local physician, Dr RINGER, who was an observant man, made a postmortem examination of the lungs, discovered a small parasite from which a multitude of microscopic objects escaped, and he wrote about it to MANSON who at this time was trying to find out where the embryo filariae departed in the daytime. His instinct (*vide supra*) had already directed him to the lungs and his thoughts to cases of haemoptysis when one day a petty Chinese mandarin, who had lived in Formosa, consulted him about a skin eruption. He had an unpleasant harsh voice and, when hawking to clear his throat, presently began to spit in a contemptuous manner avoiding the spittoons which were placed on the sawdust-covered floor for this very purpose. MANSON was fain to rebuke him for his bad manners when, as he wrote "my disgust and anger evaporated on seeing the sputum was tinged with blood. I transferred it to the microscope and, to my astonishment, I found, not the expected fibriae embryos, but the operculated egg of a different and, to me, quite new parasite." MANSON next wrote to Dr RINGER for further particulars of the pea-like parasite found at the Portuguese postmortem in due season the specimen was sent to Amoy and in the *sediment* of the spirit in which the fluke was preserved there lay operculated eggs identical with those seen in the mandarin's sputum. In this dramatic manner he connected the eggs in the *sputum* with the parent trematode subsequently named by COBBOLD *Distoma ringeri* but which has now been renamed *Paragonimus westermani*.

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[a] MANSON P. (1896) *Brit. Med. J.*, **1**, 641–712, 744.

It was characteristic of MANSON that when once on the trail he did not readily give up the search. We next hear of him examining the sputum of 150 individuals in vain for the eggs of the lung fluke and so he concluded that this parasite was not indigenous to Amoy and he had to obtain infected sputa from Japan. These duly arrived from his correspondent, Dr E BAELZ, of Tokio. He kept these specimens in stoppered bottles on his primitive laboratory bench and the story once more illustrates the fortuitous nature of his prime discoveries. It appears that one bottle in particular, to which he had added some fresh water escaped his notice for some 6 weeks till his attention was drawn by a most unpleasant smell which emanated from it. The surface was covered with a noisome greenish slimy growth. Being desirous of ascertaining the nature of this slime he abstracted some with a pipette and there observed for the first time the hatching of the operculated eggs and the escape of the miracidia on their apparent quest for a new host. We must appreciate that fortune guided his footsteps, because he could not possibly have foreseen what is now well appreciated that these eggs normally lie dormant in water for a month or longer before they hatch and it also illustrates once more the curious and uncanny foresight by which he had envisaged the necessity of some snail host in order to complete the life-cycle of this fluke —probably oo the analogy of THOMAS's discovery of the life-history of *Fasciola hepatica* in *Limnaea truncatula* (1883) but of which MANSON at that time (1880) could not have been aware.

But we do know from letters which have been discovered and which were inserted in his Diary that he wrote to a naturalist correspondent R. R. HUNGERFORD 21st October 1881 about freshwater snails in Hongkong and be replied to this effect on the whole I think *Melania libertina* must be your friend he is a hardy beast. Specimens of this snail were forwarded and as is now well known it was proved by NAKAGAWA* in 1916 to be the correct intermediary.

The same instinct he displayed some 20 years later in the predicted life history of *Bilharzia haematobia* †

There are other stories almost equally fascinating. That of the life history of *Loa loa* is much to the point. It was in the early nineties during his first years in London that he discovered the embryonic form of *Loa loa* in the blood of a missionary patient returning from the Congo and his curiosity was aroused by the demonstration of the diurnal periodicity of this parasite in contradistinction to the nocturnal periodicity of his first love *Wuchereria (Filaria) bancrofti*. MANSON was on the trail again. On questioning an intelligent native of Old Calabar on the habits of biting insects in his district which attacked with great pertinacity, engorging themselves with blood till they could not fly he learned that the most persistent and obnoxious was a day biting

* NAKAGAWA, K. (1916)   *Saikin Gakuzassi* 243 p. 189

† MANSON, P. (1898).   *Tropical Diseases*   1st Ed.   London   Cassell & Co.   p 501

" mangrove fly " So he wrote to his correspondent, Dr GRATTAN GUINNESS, at Stanleyville on the Congo asking him to procure some of these flies, which were forwarded in due course and were identified as *Chrysops dimidieta*. One such specimen can still be seen in the British Museum which was caught on the 16th April, 1892, in the act of biting. He therefore concluded that this insect was the natural intermediary of *Loa loa*—a hypothesis which was found to be correct by LEIPER 21 years later. To the end he maintained his interest in this parasite and I remember one particular sporting patient infected with *Loa loa* who was unfortunately a cocaine addict. The adult filariæ were constantly to be seen travelling under the skin of his hands. He had organized races between two individual worms employing pins as starting and winning posts, and had arranged for bets to be made by his friends. From these trials MANSON was able to deduce that *Loa loa* travels under the skin at the rate of 1 inch a minute

The Malaria Story

The malaria story is too long and detailed to be related in full, and it is, moreover, familiar to many but there are certain points I would like to make clear. The first is that MANSON was totally unaware of LAVERAN's discovery of the crescent in 1880 till at least some time after 1885 though from 1884 onwards we find him engaged from time to time in a quest for the *Ba illus malariae* which had been described and widely advertised by TOMMASI-CRUDELLI [*] while a belief in it had taken root as the result of studies by CUBONI and MARCHIAFAVA [†] However he appears to have seen the pigmented bodies of MECKEL, which he subsequently realized must have been subtertian crescents He always explained to me that LAVERAN had the good fortune to have caught the parasite in the act of exflagellation and thus realized that the object was alive which was lucky for the Frenchman but unlucky for MANSON, and led him to remark Had I but had the luck of LAVERAN what a swell I might have been He was influenced by what seemed to him the constant connection of malaria with stagnant water so that in 1884 we find him engaged on what DARWIN justified as fool experiments in attempting to grow something from malarial blood incubated in sterilized marsh water and subsequently administering the brew to volunteers—somewhat on the same lines that he subsequently urged ROSS to undertake in India with mosquito-impregnated water Already he was experimenting with dyes and, in the course of a quinine famine in Hongkong had prescribed methylene blue in cases of quartan malaria with apparent success as he recorded in his Diary This experience gave rise to an idea, which he subsequently frequently expressed to NUTTALL, that selective affinity of a particular dye for any particular blood

* TOMMASI-CRUDELLI   (1886)   *Reducenon des Lucen, Roma.*

† CUBONI and MARCHIAFAVA   (1881).   *Arch. f Experiment Pharmakol.*

‡ ROSS, RONALD   (1923)   *Memoirs* p. 156

parasite might be taken to indicate possible therapeutic action In this direction recent events appear to have proved that this hypothesis was not quite so fantastic as it at first appeared. But early in the nineties he was able to recognize the malaria parasite and colour it by a stain he had compounded —borax methylene blue—a combination which rendered the dye polychrome and showed up the parasites in various shades of purple and blue Henceforward it came to be known as Manson s stain and it should be considered as the precursor of the modern Leishman stain Manson s stain is still used on the Continent, and I found it in the laboratory at Nazareth in 1918 after its evacuation by the Germans during ALLENBY s drive to Megiddo Using this ⁎ stain with carbol fuchsin as a counter-stain, MANSON was the first to obtain accurate figures of the flagellated body For studying the phenomenon he devised extremely ingenious damp chambers constructed out of blotting paper and he would sit up all night observing the act of exflagellation and pondering on its significance. This practice he continued long after the solution of the malaria problem had been obtained He decisively disposed of the dying act theory which had been suggested by KOCH and others and was convinced that exflagellation represented a stage of the life history of the malaria parasite outside the human body At this period he corresponded a great deal with LORD LISTER who was inclined to draw comparisons between exflagellation of the crescent and the production of flagellated spores which constitutes a stage in the development of mycetozoa to which LISTER had paid some attention. Then on the analogy of the development of filaria MANSON formulated the mosquito-malaria hypothesis which he logically enunciated in 1894 This led to his meeting with Ross and his constant exhortation to his pupil to *follow up the flagellum* which formed the mainstay of his theory So convinced was MANSON of the soundness of this hypothesis that he applied to the Royal Society for the modest sum of £360 to enable him to proceed to British Guiana to work out his basic idea, but unfortunately this request was not granted. He was a poor man at that time with the education of five children on his hands and could not afford to leave his practice in London. From 1895 to 1898 he sustained stimulated and counselled Ross in India in his historic quest in a series of letters the like of which have seldom been seen or equalled in science. From their study we can assess the dominating part MANSON played in the eventual elucidation of the malaria problem by Sir RONALD ROSS. These letters were genuinely acknowledged by Ross as a noble series such as few men have received and at the end of a paper in 1898⁎ he wrote These observations prove the mosquito theory of malaria as expounded by Dr PATRICK MANSON and in conclusion I should add that I have constantly received the benefit of his advice during the enquiry His brilliant induction so accurately indicated the true line of research that it has been my part merely to follow its direction and in his Nobel lecture Ross said The fundamental

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⁎ Ross, R. (1898) *Ind. med. Gaz.*, **33** Dec. 451

part of MANSON's hypothesis was the close and powerful argument to the effect that the motile filaments and the parent cells from which they spring must be meant to infect the mosquito in some manner. This was more than a hypothesis — it was a great and illuminating induction. It gave the required clue to further research; and without it I am convinced that the malaria problem would not have been solved at all and we should still be engaged in a laborious search for the parasites in water and in air.'

But there is one point to which some few lines must be devoted. This was the question of bird malaria by which, as is well known, Ross brought his researches to a successful conclusion. MANSON, who had already worked with *Proteosoma* in London, urged Ross to work with birds† as much easier to handle and control than men. This led Ross to remark 'What an ass I have been not to follow your advice before and work with birds.' The final verification of the cycle of human malaria in *Anopheles maculipennis* was effected by GRASSI and BIGNAMI in 1898. MANSON was fully aware of this as he maintained most friendly relations with these Italian scientists through the agency of Dr EDMONSTON CHARLES in Rome.

A Trypanosome Story

MANSON had never visited Central Africa during his professional career but nevertheless he played an active part in the elucidation of trypanosomiasis. He had made observations on the rat trypanosome—*Trypanosoma lewisi*—in his muck room as he called his workroom in his house, 21 Queen Anne Street, in 1892–1893. In 1896 we find him writing to DAVID BRUCE in Zululand urging him to look for this type of organism in other animals as he was convinced that it would eventually be found in man and I have still in my possession a reply from BRUCE acknowledging with gratitude his advice on this point.

There is one anecdote which must be related although MANSON himself never recorded it in print. One day in 1897 he examined a Colonial Office patient from West Africa, who had an enlarged spleen and an unexplained fever. As was his invariable custom, he made a dry as well as a wet film of his blood and laid them aside till he had leisure after dinner to examine them microscopically. It so happened that Dr L. W. SAMBON was with him at the time when a small object with an undulating movement flitted across the microscopical field. They both saw it clearly, but search through the stained dry film failed to reveal anything abnormal. Still they were both excited and elated, being convinced that they had seen a new human parasite. They forthwith resolved to obtain more specimens of blood. But the patient had

* Ross, R. (1905) *Nobel Prize Essay* "*Researches on Malaria,*" *J. R.A.M.C.* 4, 480

† Letter No. 18. 12th October 1896

‡ Ross, R. (1923) *Memoirs* p. 271

gone long ago and where were they to find him ? MANSON who well knew the habits of Colonial officials, suggested the Sports Club in St James's Square as the most likely. So hailing a hansom cab they sped on their way. It was now past 9 p.m. and the porter directed them to the smoking room, where they found their patient apparently asleep in the corner. It was soon apparent that he had dined not wisely, but too well. MANSON cautiously approached him and withdrew the formidable needle he kept in the lapel of his coat. The prospective victim opened one resentful eye and jumping to his feet, brushed MANSON to one side and dashed out of the room and that was the last they saw of him. Had it not been for this misfortune he might well have had the honour of being the first to discover the human trypanosome.

The Sprue Story

Sprue was first accurately described by MANSON in 1880 and at the same time independently by VAN DER BURG. Those who have read the original must acknowledge that MANSON'S still remains the most convincing and accurate description of this mysterious disease. His pun which is inscribed on one of the pages of his Diary that the word *sprue* may eventually come to be regarded as the part participle of the verb *to spree* has become historic. It was his custom to feed his patients with liver soup in addition to the classic milk treatment of which he was the chief exponent although he casually mentioned it in *Tropical Diseases* he never published any paper on this subject or stated the reasons upon which his beliefs were founded. We do know that he derived his liver treatment from the Chinese and it came about in something like this. In 1887 he had to leave Hongkong for a period of two months on a visit to LI HUNG-CHANG who was suffering from a sublingual abscess. He left behind a lady patient with a severe anaemia which had proved refractory to iron and arsenic—the drugs most in vogue at that time—as she undoubtedly must have been a victim of pernicious anaemia. On his return to Hongkong he was greeted by his former patient who with rosy cheeks and red lips, presented the appearance of robust health. She explained that during his absence, having tried Western medicine in vain she had consulted the local Chinese joss doctor who had restored her to health by some pills the contents of which she was quite ignorant. MANSON thereupon resolved to wrest the secret from the native practitioner and with this object in view met him at a Chinese dinner party but all his blandishments were at first in vain. At last, as the guests were dispersing he blew the gaff by explaining that the capsules contained the dried liver of a dead crow. From that time onwards liver soup figured in MANSON's pharmacopoeia and thus, quite unwittingly he anticipated to some extent the epoch making discoveries of MINOT and MURPHY in 1926.

Criticisms

No account of the Manson saga could be complete without attempting to answer criticisms which have been made about certain aspects of his work. It is said for instance that he never really logically worked out the metamorphosis of the filaria in the mosquito that he left off just before the work was completed and that he failed to grasp the true significance of the transference of the parasite from one man to another that he failed to trace the filaria to the proboscis. Although an able and appreciative reviewer in the *Veterinaeus* of 1883 (possibly COBBOLD) had suggested that the full-grown larval filariae were deposited by the mosquito in the *act of biting* yet MANSON ignored this advice and did not accept it till it had been demonstrated by Dr G CARMICHAEL LOW two years after Ross had worked out the full life-history of the malaria parasite in the mosquito He had persisted in his views that the infected mosquitoes fell into water and that the disease was transmitted by this medium. The explanation to me seems to lie in the complete lack of knowledge of the life-span and habits of mosquitoes at the time MANSON was at work in Amoy in 1877 when nothing much was known of these insects. No work of any importance existed. It was thought that, like the mayfly their life was ephemeral and that it was impossible to keep them or breed them in captivity We do know that MANSON attempted at various times to obtain some literature on these insects and wrote to the authorities at the British Museum for information on this point After a lapse of 6 months he received a reply to the effect that no such book existed, but they could supply him with one on the anatomy of the cockroach if that would suffice !

Many too have failed to appreciate his attitude of mind in the face of hostile criticism which was rife at the time of Ross's great discovery He resolutely refused to reply or to justify himself and maintained a stony silence

What I have done, I have done, he said, I look forward and never look back. This was a noble attitude of mind, a lofty attitude which brushed aside criticism. He did not stoop to recriminations and he was never heard to deprecate those who somewhat unjustly (it appeared) had attacked him.

It is said that he failed because he was not a trained zoologist he was a medical man dabbling in the realms of zoology which he had no right to enter But it must be admitted that, zoologist or no zoologist, he instigated trains of thought which few save the great masters such as DARWIN HUXLEY and WALLACE have ever been able to inspire.

Personality

In figure MANSON was more than average height, broad, robust and strong as befitted one who came of good Aberdeenshire stock and was raised amongst those northern heather-covered hills. His face was rubicund at the same time wise and jovial. He had large luminous and expressive eyes, and these,

together with his silvery hair, imparted a handsome appearance   in fact, his was such an impressive figure that his presence was instinctively sensed on entering a room.   In speech clear and incisive, in manner kind and benign, it followed that his lectures were models of lucidity   He was no pedant, but a very human human being   To some his florid appearance suggested that of a sporting hunting squire (as Dr H B GUPPY expressed it) rather than that of a palefaced student engaged in solving great medical problems, sickled o er with the pale cast of thought   He was fond of sport   in youth he had been a good cricketer   he was an exceptionally fine shot.   It is said that on one occasion when he was eighteen, he took out forty cartridges returning with thirty nine partridges and one unspent cartridge   He was an unusually keen fisherman, a devoted disciple of Izaak Walton.   These traits, inherited from youth, he maintained into old age   so that he spent his years of retirement pursuing his favourite sports   His one disability was gout, from which he began to suffer in 1886 (at 43 years of age) and which crippled him entirely for weeks   sometimes for months, so that it was a constant source of wonderment how   in spite of this handicap   he managed to get through such a stupendous amount of work.   Though normally of even temperament, these recurrent and painful attacks rendered him at times a little irritable but throughout, his scientific enthusiasm showed no signs of flagging   He had early decided that dietetic precautions were of no avail in his particular case as relapses occurred in spite of what he ate or drank   One day   several years after his death   an old patient of his consulted me and   after relating in detail his signs and symptoms   asked my opinion.   Well, I said   I think you must have the gout.   That is true, he answered   for your father in law was exactly of the same opinion   he prescribed some mixture, but handed me a formidable list of dietetic and alcoholic restrictions.   Thank you Sir Patrick, the patient replied,   I will certainly do what you direct, but you have been a trifle hard on me and,   he added,   you do not look very well yourself this morning Sir Patrick.   He had a flushed face and was sitting back in his chair with his right foot swathed in cotton wool propped up on a chair   No   MANSON answered,   you are quite right, I am indeed far from well, as I have a most damnable attack of the gout, but (with a somewhat wicked wink) that does not prevent me from drinking my glass of port every night.   Even when so crippled, and his hands so shaky that he could hardly grasp a rod, nothing could dissuade him from fishing his favourite loughs   Till he was over 60 he travelled yearly to his native Highlands in pursuit of grouse and black game, and once when severely incapacitated in Ireland in 1909 he was wheeled to the edge of the moor in an invalid chair   A pack of doubly wary Irish grouse was driven over his head, and, with unerring aim, he picked out a right and left and these   I believe, were the last shots he ever fired.

MANSON was fond of animals and flowers   He was a good gardener and never so happy as when pottering about with his roses.   He was also a good

carpenter completely at home at his bench. It was natural that children should be attracted to him as he was to them, and many hours were agreeably spent with his grandson in his boat on some Irish lough pointing out geological features, wild flowers, birds and even formations of rock and clouds. On all these he had something of interest to impart.

On one occasion, on being asked to explain the nature of rain he gave an extempore lecture on vapour tension and other technicalities of meteorology (for it should be remembered that he once had his own meteorological station in Amoy) when he was interrupted by his grandson who wished to know why it could not rain in a reverse direction ? To which, for once, he failed to provide an adequate answer.

As an after-dinner speaker he was excellent. He usually adopted the most correct ladies' English but if the occasion demanded, he could lapse into broad Scots, his language being well flavoured with Attic salt.

It is said that sometimes at public dinners he was consulted by high-placed ladies regarding their digestion and that his invariable reply was: "If I were suffering like you I should take a large dose of castor oil." On occasion he could exhibit a merry wit. On the turning of a phrase or emphasis on a word he was a pastmaster as the following story shows. Called into consultation in the North of England to the sick bed of a well-known peer he arrived there after a long journey from London and made the diagnosis of typhoid fever which had not so far been suggested, but which ultimately proved to be correct. Although it came to his knowledge that four other doctors had been called in and had each been paid a fee he found that he had been neglected and his account was never settled. At length he received from the lady of the house a small volume of verse as his recompense with the inscription: "To one who succeeded where others failed," which he deftly converted to "one who failed where others had succeeded."

There was a time when he was publicly nicknamed "Mosquito Manson," and cartoons appeared in the evening papers of his transformation into some species of anopheles. One evening whilst walking down St. James's Street three well-known physicians appeared at the door of their club. On seeing his striking figure ambling past one enquired who he might be. The reply was "Mosquito Manson" and pointing to his forehead insinuated that he was somewhat odd. Manson sensing this, turned round and reciprocated the gesture. Events proved that he was not quite so mentally deranged as they had insinuated.

Manson was a great admirer of the Chinese. He knew something of their language and was familiar with their customs. A Chinaman could always count on his sympathy. In his later years, when visiting his wards, a smile of recognition always appeared when he spotted an Oriental patient in one of his beds. Sidling up to him, he would murmur weird noises into his ear. The Chinaman would shake his head—probably he was using the wrong dialect—and would answer "Me no savvy—you speakee English."

MANSON was a master of microscopic technique. He would take great trouble in arranging the condenser and illumination and he always succeeded in procuring the best definition with the fine lenses he employed. But the microscope itself (an old Watson model) appeared to the casual observer a most rickety affair. NUTTALL and other microscopists of fame invariably commented on this and wondered how he ever managed to see anything at all with such a primitive tool.

Of his precepts what better advice could be given to any young man than these lines he wrote in my notebook in 1909. Never refuse to see what you do not want to see or what might go against your own cherished hypotheses or against the views of authorities. These are just the clues to follow up, as is also and emphatically so, the thing you have never seen or heard of before. The thing you cannot get a pigeon hole for is the finger-point showing the way to discovery.

To quote the stirring lines of MANSON'S obituary notice in *The Times* "He founded and inspired the great band of British workers by whose efforts the tropics have been made safe for the white man. Triumphs over a whole category of diseases have proceeded naturally from his teaching. How great that service was this generation is probably incapable adequately of judging but our children's children may understand the full significance of his labours that, whatever betide, will stand as a memorial for all time, a gift to humanity, the value of which must increase from generation to generation.

It is not too much to hope that as a tribute to this great man this Society may consider at some time in the future the establishment of a Manson Scholarship as expressed in a last document he penned almost with his dying breath.

OTHER TRIBUTES TO MANSON'S LIFE AND WORK

Dr G. Carmichael Low. Sir PHILIP MANSON BAHR, in his excellent address, has given us many details of MANSON'S life and discoveries in the Far East. It is not necessary therefore for me to go into these again. Suffice it to say that it is a marvellous history. a young man going out to a new country with no previous teaching of the diseases he would be likely to meet with. Many times in after life he confided to me how much he would have valued a course on tropical medicine before going to the East, what hours of time and labour it would have saved him and how much more work he could have accomplished. Yet—and this is where the stroke of genius comes in—he overcame all difficulties, mastered the subject by himself without any

teaching and by his discoveries laid the foundation of modern tropical medicine. The long tedious hours spent in study and research by himself in China were never forgotten as we shall see when he returned to England. There were no specialists out there to appeal to and so he had himself to act as physician, surgeon and gynaecologist and in all these branches he showed first-rate ability. With so much in hand it is wonderful that he was able to do scientific research work and make the discoveries in parasitology that he did.

He came home in 1889 with the intention of retiring, but the fall in the Chinese dollar (a fortunate circumstance it turned out to be) compelled him to do further medical work, so he came to London in 1890 and settled at 21 Queen Anne Street and began practice again. In 1892 he was appointed to the staff of the Seamen's Hospital at Greenwich and in 1894 he started lecturing and giving instruction in tropical diseases in London. Not long afterwards in July 1897 he was appointed Medical Adviser to the Colonial Office as successor to Sir CHARLES GAGE BROWN and this fortunate appointment gave him the great chance of his life by bringing him into intimate contact with Mr JOSEPH CHAMBERLAIN.

In November 1899 I first met MANSON. I had just come home from Vienna and, armed with an introduction from Professor ROBERT MUIR, of Glasgow, I called at 21 Queen Anne Street. The malarial problem had just been settled, but filariasis still remained. After a talk it was decided that I should take this up and should work on filariated mosquitoes which the younger BANCROFT had sent to MANSON from Australia. After mastering the literature of the subject and getting up all that was known about mosquitoes, I would then go to the newly opened School of Tropical Medicine at the Royal Albert Docks and work there.

In my Presidential Address to the Society of Tropical Medicine in November 1929 I dealt fully with the foundation of the London School of Tropical Medicine a new departure which, as Sir PHILIP says, set the seal on MANSON's fame. I shall not go into full details again here but shall just mention the salient points. I have already said MANSON had begun lecturing and giving instruction in tropical diseases in 1894 and as time went on he realized more and more the necessity that medical men, outside the Army and Indian Medical Service should have special teaching before proceeding abroad. CHAMBERLAIN was at once struck with the idea, circularized the Governors of the Colonies and wrote to the Board of Management of the Seamen's Hospital Society about the matter and the latter acceded to his request that the school should be located at the Albert Dock Hospital in the East-end of London. MANSON had his way therefore, but it will astonish the younger generation to bear that there was opposition of a most hostile kind to the whole scheme. Fortunately a strong man was in power and CHAMBERLAIN swept aside the objections, stated that he had complete confidence in MANSON's advice and insisted on carrying the matter through. At the same time through the energy

of ALFRED JONES and RUBERT BOYCE, the Liverpool School was founded. Success attended both Schools, and other nations soon followed the lead of England by establishing similar institutions. Time will not permit me to follow up the history of the Schools further the old London School of Tropical Medicine ultimately became incorporated with the London School of Hygiene and Tropical Medicine and is still thriving as is also the Liverpool School.

I was abroad from 1901 to the end of 1902 and then succeeded DANIELS as Superintendent of the School where of course I came into close contact with Sir PATRICK. The School was his child and he was devoted to it. Though often physically unfit, he never liked missing his bi-weekly visits to the Hospital, and many times made the long journey to the Albert Docks when he should really have been in bed After a lecture the wards of the Hospital were visited and clinical instruction given These visits were eagerly looked forward to by the students and staff and as the Hospital and School were adjoining no time was wasted in travelling There was accommodation for a few students to live in and the proximity of the two buildings allowed of nightly visits for studying interesting cases and taking night bloods Sir PATRICK was an accurate and painstaking physician sympathetic and kindly to his patients and an excellent diagnostician Many were the interesting cases gone into with him. The buildings small at first, quickly expanded, and the Hospital was enlarged by the addition of two up-to-date wards For the members of the staff and younger workers MANSON considered that it was essential that they should go abroad and win their spurs and so many expeditions were always on foot After the malarial problem had been solved he thought a practical demonstration would help so he devised the Roman Campagna expedition. SAMBON and I in 1901 lived in a mosquito-proof house throughout the malarial season without any bad effects and we sent infected mosquitoes back to England which gave malaria to two volunteers. After some 5 years it was felt that the work of teaching the different subjects was too much for one man so Departments of Helminthology and Protozoology were founded Dr LEIPER was appointed in January 1905 and Dr WENYON later in the same year

In 1907 following a suggestion originally made by Sir JAMES CANTLIE, the Society of Tropical Medicine and Hygiene was founded and Sir PATRICK was of course elected as first President. For those specially interested, the history of the foundation will be found in the TRANSACTIONS * The Society was a success from the beginning and has never looked back at any time of its career

It met for years at the rooms of the Medical Society of London in Chandos Street, but it was felt that it should have a house of its own and the building we are now sitting in was acquired in 1931 and formally opened by the PRINCE OF WALES on 17th March 1932. In honour of Sir PATRICK MANSON and to perpetuate his illustrious memory it was named MANSON HOUSE.

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* Low G CARMICHAEL (1928) *Trans R. Soc trop Med. Hyg.*, **22** (2) pp 197-202.

I have many personal reminiscences of SIR PATRICK, from the little laboratory in the top story of 21 Queen Anne Street to the Tropical Hospital and School at the docks, then to his retirement up to his death. In the old days I often stood by in his attacks of gout and had many arguments with him on the effect of diet and especially port wine but he just laughed and said " Low I don t believe they have anything to do with it." In these days SIR PATRICK and Lady MANSON had a house at Chalfont St. Giles, where they spent week-ends and where I often went. He was a skilled carpenter and spent much of his time after he became crippled with his gout in working at different things which required such skill his garden was also a source of pleasure to him. The crippling put an end to walking, but in Ireland the fishing, of which he was very fond, could be done by boat, and many times I took part in expeditions on the lakes with him. Well do I remember the day when a big salmon broke away from his line, his disappointment, and the gradual rise in the estimated size which went on for several days after One day the farmer said to me you should use a fly called the Fiery Brown," so I said you might bring me some the next time you go to Galway He did so and I told SIR PATRICK. He laughed and said he did not believe in special flies, but I noticed that he took one with him the next time he went out and, would you believe it, caught three salmon with it.

I often went to see him during the last war and after it, and a fortnight before his death on 9th April, 1922, found him planning a new Filariasis expedition to the Pacific.

Yes MANSON was a genius and had a wonderful brain, and it is right that he should take his place with LISTER, PASTEUR and KOCH the great men of medicine, as pictured in *The Times* of Monday 21st March, 1932. Our Society will perpetuate his name to all time and SIR PHILIP s suggestion that as a further tribute to this great man the Society may at some time in the future consider the establishment of a Manson Scholarship is an excellent one. He spoke to me about this some little time before his death. It was a sad moment for me to shake his hand for the last time after knowing him so long and so intimately *Requiescat in pace*

Dr H M Hanschell   Mr PRESIDENT   During Sir PATRICK MANSON'S last year (1910 to 1911) of tenure of office as Physician to the Albert Dock Hospital to which his School of Tropical Medicine was then linked and in fact contiguous, I had the privilege of being his House Physician. Sir PHILIP has told us about MANSON s genius and the great qualities shown in action of his mind and heart. I am conscious that I am qualified to speak of MANSON only on a humbler plane and though listening to Sir PHILIP has brought up in my mind vivid and happy memories of my illustrious chief, I fear that it is beyond my powers to portray in words more than the mere semblance of MANSON's personality and ways, which won the loyalty and affection

and inspired the mind and actions of the young medical men whom he welcomed and taught at his hospital and his school. Sir PATRICK was always considerate to me and both at hospital and at those highly prized occasions of his and Lady MANSON's hospitality at their home in Queen Anne Street, I received much kindness and encouragement from both—and all with a very charming informality and dignity

Sir PATRICK was great in mind and great in body. In those days he was stricken in body and it was obviously often painful for him to walk—yet he hardly ever missed his hospital visit and never his school lecture. He came down to hospital by car with drawn blinds—drawn not because of the unattractive streets he must pass through but because he smoked his pipe on the way—and it was not the thing in those more formal days for Aldermen to be seen smoking pipes in public places. Sometimes when the car halted in the hospital yard its blinds stayed drawn and its doors stayed shut—the pipe was not finished. When he drew the blind up you could go forward and help him alight. With the aid of a stick he would hobble from bed to bed, or be wheeled in a chair. He always seemed eager, in good form and smiling—his black eyes bright, kindly and at times piercing enough. The strength and liveliness of mind gained it seemed from the infirmity of body—the great body and the greater mind. Sir PATRICK looked and was benign and wise. His talk at the bedside was mostly grave and kindly though often salted with a pleasant astringent humour. His shaky hands shaking like that for many years did not prevent his percussing a chest for he did astonishingly well by a sharp flick of the forefinger off the mid which compelled the back of the forefinger to sharp impact on the chest wall—his physical infirmity could not dismay him. His preference for his Chinese patients was marked and openly avowed. His face lit up with pleasure at the sight of them and he approached them with queer noises which he, MANSON said was Chinese. The always polite Chinese patients smiled and bowed in response but reverted to tolerant and courteous silence, and it must be said they never tried *their* Chinese on *him*.

I think MANSON shone at his best in the diagnosis and treatment of liver abscess and in the management of his sprue cases. In those days we had always three or four liver abscess cases with us. There was never any hunch nonsense about his diagnosis of liver abscess. He said the secret was always to think of it, and he used to track down step by step and bit by bit the evidence, predominantly clinical and bedside which led him to the diagnosis, to he proved accurate soon after at aspiration of liver.

MANSON would tell us who listened to him, that we ought to adventure serenely—we should not bother too much about what in any case was far ahead—we should cast our bread on the waters that it might come back to us with increase after many days

I remember it was a happiness to have worked for him and with him

One of the Founders of the Liverpool School, Sir RUBERT BOYCE, wrote of those who followed after MANSON as the New Conquistadores of the Tropics I venture to add and truthfully I am sure that MANSON was their COLUMBUS.

Lieut Colonel S P James   This is a great occasion   it is an occasion on which we are greatly honoured in honouring the memory of a great man —the father of tropical medicine   Sir PHILIP in his very interesting address spoke of the inspiration encouragement and practical help which Sir PATRICK MANSON gave to younger men in the tropics who had decided, for one reason or another to renounce the financial and other attractions of a practising doctor's life in favour of a career of medical research—a career which some of us who are here this afternoon know only too well often winds uphill all the way It is because during my early life in India, I was one of those young men whom Sir PATRICK encouraged and guided that I wish today to acknowledge my indebtedness to him and to express my profound admiration of all he said and did.   In this connection I was glad that Sir PHILIP recorded in his address one of the many tributes that Sir RONALD ROSS paid to Sir PATRICK's inspiration, encouragement and guidance—I mean the tribute in which Ross said that the many letters which MANSON sent to him between 1895 and 1898 were a noble series unequalled in scientific literature and such as few men had had the good fortune to receive.   As regards myself Sir PATRICK began to encourage and to help me first in 1899   I had written to him from Travancore in Southern India to tell him of my work on mosquitoes and filariasis, but I did not have much hope or expectation that he would have time or inclination to reply I had never met him up to that time so I did not know what I learned later namely that he was perhaps the most generous and unselfish research worker the world of Science had ever seen.   The reply I received marked a turning point in my career because from that moment I decided to devote myself henceforth to medical research alone.

About a year later I received another proof of Sir PATRICK's great generosity to workers in far distant lands when I read in the *British Medical Journal* for the 1st September 1900 what he had said at the British Medical Association about my observations and those of Dr G C Low   Nothing could have given me greater encouragement than the knowledge that he had stated publicly that I shared with Dr Low the merit of having made an important contribution to tropical medicine   It was not until 7 years later that I met Sir PATRICK personally for the first time, and that was when I got my first leave home to England.   He was then at the zenith of his fame in London but nothing could exceed his kindness and that of Lady MANSON to all workers from India and all parts of the British Empire.   Some may remember that it was quite a red-letter day to any of us when we lunched at 21 Queen Anne Street, and went with Sir PATRICK in his car to the Albert Dock Hospital in order to listen to his bedside talks and lectures.   At the beginning of the present century

one of the subjects that interested Sir PATRICK very much was the projected Panama Canal. He used to tell me long before the canal was built, that India should be careful lest this canal might introduce yellow fever into her seaports. I have always been glad that his views on that danger were so firm, because the Indian Government were so impressed by them that in 1910 they were good enough to send me round the world via Panama to study the endemic and epidemic areas of yellow fever on the spot. After I returned I had the pleasure of meeting Sir PATRICK and Lady MANSON in Ceylon amid the domestic scenes of which Sir PHILIP has shown a picture. Those and many later memories are very precious and I feel that the pleasure one derives from them will be shared by many others, especially by those who happened to be working in the tropics at the time when the science of Protozoology was in its early infancy and when the science of Medical Entomology was yet unborn.

Professor Gordon King I count it an unusual privilege to be able to be present at this meeting and say a word on behalf of the medical profession in Hongkong. I listened with interest to Sir PHILIP's description of the early days of Sir PATRICK in Formosa Amoy and Hoogkong and incidentally I should like to assure Sir PHILIP that the Chinese characters on the letter he showed us were the right way up and the right way round! Sir PATRICK left an indelible mark in Hongkong his name is still something of a legend in the colony. Perhaps the most permanent thing he left was the Medical College which he was instrumental in founding. He and Sir JAMES CANTLIE in 1886 founded the Hongkong College of Medicine the first class consisted of six or eight members one of whom was the late Dr SUN YAT SEN. The main emphasis was placed on clinical work, and the original teaching hospital still survived in 1941 as the Nethersole Hospital of the London Missionary Society. In 1911 the Hongkong College of Medicine expanded into a University with Faculties of Medicine, Arts and Engineering and continued with increasing prosperity up to the time of the outbreak of the Pacific war. At the present time one-half of the student body from Hongkong is in Free China, whither the students migrated to carry on their studies. The firm of practitioners that MANSON founded still survived in 1941 and the doyen of the profession in Hongkong was Dr G. D. R. BLACK the senior member of the firm. When the war broke out he became a temporary Colonel in the R.A.M.C. and was in charge of an emergency hospital at Stanley one of the last points to resist in Hongkong. The Japanese, after severe fighting and many losses got to St. Stephen's College Stanley early on Christmas morning. Dr BLACK went to the door of the hospital, pointed to the Red Cross and said that only wounded men were inside. The Japanese reply was to plunge a bayonet through his chest. Then they bayoneted Captain WITNEY who stood beside him, and about fifty helpless wounded as they lay in their beds. Another though minor tragedy of the war was that the first Diploma course in Tropical Medicine in Hongkong was

interrupted by the fighting   It probably is not known to many of you that a Hongkong Diploma in Tropical Medicine and Hygiene was inaugurated in 1941   I think I have the only syllabus of the course to reach this country in my hand.   There were eighteen candidates and the course was a 6 months' one, but it was brought to an untimely end by war when less than half completed.   I must not say any more except to express once again my appreciation of the opportunity of saying a word or two at this historic meeting

The President Sir Harold Scott   I was going to say a few words but will forbear as the hour is late   This is of course a very notable day for us. It is notable for three reasons   first of all, it is the birthday of HIS MAJESTY THE KING our PATRON   next we are celebrating the centenary of the birth of Sir PATRICK MANSON and lastly we have had the privilege of listening to the Saga by Sir PHILIP MANSON BAHR, who is, I suppose as widely known round the world as was his father-in-law in his day   There is nothing for me to add to what we have all heard only this that MANSON has left a memory enshrined and enthroned in the hearts of his many pupils   for such as this there is no death.

A number of lantern slides were shown at the meeting and there were exhibits of MANSON's original drawings and preparations demonstrating his chief scientific discoveries.   MANSON's Diary was also on view

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Transactions of the Royal Society of
Tropical Medicine and Hygiene.
Vol. XXXVIII No 6 July 1945

COMMUNICATIONS

FURTHER PROGRESS IN THE CONTROL OF SLEEPING SICKNESS IN NIGERIA.

BY

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Earlier papers (LESTER, 1933 1938 1939) described the work of the Nigerian Sleeping Sickness Service. Reasons were given for the adoption of the survey and mass treatment system and for the establishment of a control section, financed by the Colonial Development Fund, for measures directed against tsetse fly The present paper brings these accounts up to date

At the beginning of the war three out of the six treatment teams were broken up to release staff for war service Late in 1940 the whole service went on to a maintenance basis In spite of this, progress has been maintained and there has been much general improvement. Fortunately nearly all new areas had been surveyed. The skeleton staff remaining was able to cope with those resurveys which were urgently needed. More reliance had to be placed on the establishment of sleeping sickness dispensaries as permanent treatment centres. For a time development work in the sleeping sickness settlements was slowed up Later by redoubling their efforts the limited staff available were able to make further progress and even to start work in new areas.

Before describing what has been done it would be as well to give a description of the characteristics of the disease and its general distribution

CHARACTERISTICS OF THE DISEASE

In earlier years medical officers toured the few sleeping sickness areas then known to treat such cases as cared to come in to them voluntarily. It was easy to fit patients into the classical picture of gambiense sleeping sickness. Many complained of sleeping and showed signs of advanced nervous disease. Others coming for treatment of other complaints and found to have trypanosomiasis without much in the way of symptoms were thought to be early cases.

Once mass survey of the whole population was started difficulties arose. In most areas the proportion of advanced nervous cases was very small, usually less than 2 per cent. Only by careful questioning could the majority of patients be made to admit to occasional bouts of fever and headache. If they complained at all, it would be of general weakness. Signs of nervous involvement were rare though up to 50 per cent of such mild cases might show minor changes in the cerebrospinal fluid.

Some of the worst cases might have attended treatment centres voluntarily and others might be missed through people refusing to bring them in at the time of the survey. At one time sleeping sickness staff had to contend with a widespread prejudice. People believed that the disease was contagious. They only recognized it in the sleeping stage and feared it greatly. Known cases were shunned by all but their immediate relatives. As a result patients often would hide their illness if they could. Making due allowance for these factors it was still difficult to make the survey findings fit the classical picture. It was impossible to believe that year after year the survey teams always arrived in a district in time to catch the great majority of patients as early cases. It became clear that in many parts of the country these early cases were the real disease and that most of them would never go on to the third stage.

There are great differences in the disease in different areas. In parts of Benue Province for instance, there are districts in which the disease seems to have a very low virulence. There is indirect evidence that suggests that natural recovery may not be uncommon in some of them. In other places there may be a high proportion of nervous cases, and the duration of the disease may be only 12 to 18 months. Changes in virulence sometimes occur during the course of an epidemic. There have been instances of a virulent infection superimposed on the mild form and of the mild form occurring after a virulent epidemic had burned itself out. It is this element of uncertainty which makes it inadvisable to leave any large number of cases untreated just to obtain further information about natural recovery. In a mild area of Benue Province a 100 or so cases are being left untreated and are being kept under regular observation. The clinical findings will be of great interest though they can hardly be conclusive without laboratory confirmation in the way of blood cultures and animal transmissions, which are impossible at present in that part of the country.

In some districts there was much depopulation and only repeated resurveys and protective measures succeeded in putting a stop to it. In the Rukuba area of Plateau Province people were so shy and suspicious that a survey was impossible for a number of years. To try to break down this prejudice a dispensary was established. Many hundreds of patients came in for treatment but hardly any of them would attend long enough or regularly enough for cure. As a result large numbers of them died.

It is convenient to divide cases into three types.

(a) The commonest is the mild form. The case is diagnosed by the presence of trypanosomes in enlarged cervical glands, or in blood. He often has a characteristic puffiness about the malar region of the face. It appears that after initial fever and headache the disease and his resistance to it reach a state of equilibrium. He suffers from occasional attacks of headache and fever and from a certain amount of weakness. Such patients are below par mentally and physically and may remain in this state for years. They usually have a lowered resistance to other diseases. In some of the more heavily infected localities it is this increased susceptibility to intercurrent disease, particularly bronchopneumonia and dysenteries that causes an abnormally high death-rate and consequent depopulation.

(b) The second type is much more rare. Toxaemia is the salient feature. Patients may complain of headache, fever and weakness. Oedema of the limbs is common. There is often considerable emaciation. Progress of the disease may be rapid, untreated patients dying in an attack of acute toxaemia with high fever, vomiting, etc.

One such case recently occurred in Kaduna in a European Catholic sister at the Convent School. The writer is indebted to Dr H B Lee for permission to quote the case. The patient was first seen after she had been gravely ill for 3 days with acute toxaemia. She had no definite history of previous illness. When admitted to hospital she had a temperature of 105 0 F. She had large raised purpuric patches on her legs, and was vomiting continually. She was already very dehydrated. A massive trypanosome infection of the blood was found at routine examination. The first full dose of antrypol brought the temperature down to normal and stopped the vomiting. She was given a full course of treatment. All symptoms, including the rash, disappeared and she made an uninterrupted recovery.

(c) The third is the nervous type of case. The signs of progressive involvement of the central nervous system are characteristic. The earliest signs are often slight involuntary movements of the hands and fingers coupled with slight unsteadiness and an alteration in gait. Patients complain of sleeping more than they should in the day time. Changes in nutrition are common. Wasting may be extreme though a puffy obesity is not infrequent. Unless carried off by intercurrent disease the patient reaches the characteristic sleeping stage. Varying degrees of mental aberration up to acute mania are common.

The proportion of patients suffering from these three types varies very greatly though even in the more virulent epidemics the mild type (a) always seems to be in the majority.

DISTRIBUTION

The history of sleeping sickness in Nigeria dates back to the times of the slave traders. In parts of the Northern Provinces there are said to have been a series of small local epidemics at intervals of 15 years or so. Heavily infected villages soon fell a prey to their enemies. Such people as did survive were carried off as slaves by their stronger neighbours.

Prior to British rule both Muslims and pagans lived in comparatively large walled towns and villages for safety. The land close to their towns was farmed extensively. This sufficed to keep back tsetse. Once the pressing need for protection disappeared people moved out of the towns in search of more fertile land. Many of them now live in small scattered hamlets. The amount of land they farm is not sufficient to protect them from tsetse fly which often spreads right into their villages during the rains. It is this change in the habits of the people which makes them more exposed to the attack of tsetse. This, together with the spread of infection by movement of large numbers of labourers, in building roads and railways and later by improved transport facilities generally led to a great increase in infection.

The correlation between the present areas of infection and the lines of communication, railways roads and mining areas is very striking. The main belt is confined to the central part of the country including practically the whole of Zaria, Niger and Benue Provinces and the southern parts of Katsina, Kano Bauchi and Plateau Provinces. It extends in the form of a tongue to the north-east, following the line of the rivers of the Chad basin.

At the edge of this belt the incidence is much more sporadic. Occasional patches of heavy infection are found in districts otherwise free. The peripheral parts of the Northern Provinces areas which from their position are more isolated from contact with the rest of the country are still practically free of infection in spite of being heavily infested with tsetse fly.

In the Southern Provinces there are isolated foci in Owerri and Ogoja Provinces and in the Cameroons. Some infection has recently been carried to the Ife Ilesha gold mining areas of Oyo Province. Little if any infection is reported from the greater part of the south.

METHOD OF CONTROL

In 1930 once it was realised that the voluntary system of treatment had failed to check the spread of the disease, a start was made in the training of Africans to act as microscopists and to give treatment. The system of compulsory surveys and mass treatment was started then and gradually expanded.

The original intention was to control sleeping sickness by examining the whole population of the infected areas once a year All old cases were to be kept under regular observation and a further course of treatment given as required

This is the system in operation in the neighbouring French and Belgian territories. At the time of the writer's visit to the Belgian Congo in 1939 the aim was to examine all the population in sleeping sickness areas twice a year Every man, woman and child was examined for sleeping sickness and for obvious signs of yaws leprosy and syphilis Suspects were examined for tuberculosis and all males for gonorrhoea Massive registers had to be kept for each village Every householder had a bulky passport on which his medical history was endorsed The results of all examinations of himself and his family were entered in the village register and on his passport No one from a sleeping sickness area was allowed to remove out of his district without the permission of the local district officer On arrival at his destination he had to report to the new district officer and have his passport endorsed accordingly

The survey work was done by European *agents sanitaires* each with four or five native assistants These teams would examine about 300 people a day They were under the control of the local medical officer The sleeping sickness cases found were treated at dispensaries and hospitals Each case was re-examined several times a year and given further treatment if necessary Treatment was controlled by cerebrospinal fluid examination. Once the cerebrospinal fluid became normal the criterion of cure was that it should remain so for four further half yearly examinations

The writer had an opportunity of seeing this system of operation It was most impressive It has many advantages medically But it needs a very large staff at one time 180 medical officers and 280 *agents sanitaires* were engaged chiefly on this work In 1937 some 5 034 442 people were examined and 14,921 cases found. In addition 50,980 old cases were under control

It soon became clear that the sleeping sickness areas in Nigeria are too extensive for such a system to be practicable Infection rates of 5 to 25 per cent. were general in most of the central parts of the Northern Provinces. The Muslims are great traders and travellers in this country A passport system with similar restrictions would entail a complete change of life of much of the population. To enforce it an army of officials would be necessary together with a degree of compulsion foreign to British ideas of colonial administration. The special medical staff required would be greater than that available at present for all general medical services The cost would be out of all proportion to the resources of the country

In view of the mildness of the disease in many areas such an effort would hardly be justified. This must be regarded purely as a question of public health. In areas where the disease is very virulent and the great majority of cases are likely to die in a short time, without treatment, much compulsion and continued intensive treatment is essential In other areas once the incidence

has been reduced to reasonable limits and adequate information obtained to plan control it is sufficient to establish permanent treatment centres. Attendances at these are voluntary. Local spot resurveys can be carried out from them. These, together with the numbers of sleeping sickness patients attending voluntarily, give an idea of what is going on in the district. In most areas where infection rates have been reduced to the neighbourhood of 1 per cent the disease has very little effect on general mortality. Providing that population figures are satisfactory, that there are no signs of any increase in the disease and that permanent treatment facilities are available the great effort needed to reduce the incidence still further by mass treatment would be out of all proportion to the benefits to be obtained.

The Nigerian policy is, then, to establish a convenient dispensary or dressing station once the initial survey has been completed. The information obtained at the survey makes possible the planning of effective protective measures. In virulent epidemics several complete resurveys may be necessary though usually the occasional spot resurvey of some of the worst villages are sufficient. No restrictions on the general population are enforced. In a few areas where there is a gradually increased risk of acquiring the disease through their occupation mines labourers have to be controlled.

Therapeutic Measures.

Mass Treatment

The peacetime organization for survey and mass treatment consisted of six sleeping sickness teams. Each has twenty-four trained Africans skilled at using a microscope and giving treatment. Their work is supervised by a non-commissioned officer seconded from the R.A.M.C., assisted by two African male nurses. One sleeping sickness medical officer takes charge of two teams.

Before the survey of a district is started a number of temporary shelters are put up at strategic points to serve as examination centres. Arrangements are made to call in 500 to 1 000 people a day from neighbouring villages to each centre in turn. At the start of a day's work the people are lined up village by village and their names checked against the census records. Then each is examined for enlarged cervical glands. Special attention is paid to all people who look ill or whose general appearance is in any way suspicious of sleeping sickness, whether they have enlarged glands or not. Fresh gland preparations and stained blood films are examined for all suspects. A team usually has a dozen microscopes in action. In some localities the proportion of cases diagnosable by blood examination alone is so low that medical officers may prefer to do repeated gland juice examinations and leave out blood examination with the exception of suspects without enlarged glands.

At the end of the day all cases diagnosed are given a trial dose of 0·2 gramme of antrypol. This was found to be necessary because occasional

patients have a marked idiosyncrasy to the drug some little time after injection they collapse become unconscious, and may cease breathing When the use of Bayer 205 and antrypol first became universal such alarming cases of collapse occurred about once in every 2,000 cases. The more severe of them became practically pulseless. In one or two instances, patients died, in spite of artificial respiration and injection of cardiac stimulants The preliminary injection of a fifth of the normal dose is sufficient to show up this idiosyncrasy in a milder degree. Susceptible cases can be weeded out and given tryparsamide only It is very rare for the ill effects of the drug to be at all severe with the trial dose, though several cases have occurred Two or three cases of death after the trial dose have been reported from dispensaries where patients have wandered off to the market immediately after receiving the injection, instead of resting quietly for an hour or so as instructed

When the survey of a district has been completed the team is split up into small sub-teams to give treatment. Up till the end of 1934 treatment was by tryparsamide alone, some 25 to 30 grammes being given in 2 gramme doses at 5-day intervals Work at the Gadau laboratories showed that by giving Bayer 205 or antrypol for the first three injections the course of tryparsamide could be reduced very considerably The standard form of treatment was changed to three 1 gramme doses of antrypol followed by five 2 gramme doses of tryparsamide This although rather less effective for advanced nervous cases than the prolonged course of tryparsamide, sterilizes the mild cases found by the teams more rapidly A great advantage is the shorter period of treatment, only eight injections being required instead of fifteen Work is speeded up It is more popular as it means less interference with the general life of the community undergoing treatment.

On the whole the incidence of ocular trouble and dermatitis is about the same with both courses of treatment. Usually these complications are comparatively rare though some tribes seem to be more susceptible than others The sterilizing effect of antrypol is very much greater than that of tryparsamide The trial dose of 0 2 gramme of antrypol or Bayer 205 will sterilize both blood and gland juice temporarily This has an important hearing on the question of drug resistance The first doses of antrypol cure a considerable proportion of the milder cases and sterilize the peripheral blood of the remainder The subsequent injections of tryparsamide have their effect on trypanosomes in the central nervous system Undoubtedly a proportion of the more advanced cases will relapse and will subsequently require dispensary treatment. The standard treatment there is 3 grammes of antrypol and 17 grammes of try parsamide When necessary the amount of tryparsamide is increased still further

There has been no evidence in Nigeria of tryparsamide resistant strains having been produced as a result of mass treatment In two epidemic areas, Ganawuri and Abua, large numbers of patients did become resistant to treatment. In each case the damage was done before a complete survey could

be carried out. It was caused by patients attending voluntarily for a few injections of tryparsamide and then going away as soon as they felt better. Two or 3 months later they would relapse and come in for more inadequate treatment, and so on. At that time it was impossible to compel them to attend regularly with the result that many became completely resistant. Fortunately there was no evidence of the spread of the resistant strains to new patients. Working with experimental animals infected with Nigerian strains isolated from man, considerable numbers of injections of tryparsamide are needed before there is much increase in drug resistance. By then the strains will have become almost or completely non-transmissible through tsetse fly. Apparently this is what happens in man.

An additional safeguard is the fact that in most of these incurable advanced cases it is impossible by ordinary methods to demonstrate trypanosomes in the peripheral blood. Trypanosomes often can be found in the central nervous system, but are so scanty in the blood that they only show up at blood culture. The result is that the very scanty trypanosomes that do occur in the blood of the drug fast patient have such a low transmissibility or are so completely intransmissible, that there is no chance of their infecting tsetse-fly. Such patients although incurable are not dangerous to others.

The work done by the teams is shown in the following table. Up till 1935 the situation was such that it was impossible to spare staff to do much in the way of resurveys. The increase in the number of cases found by the

	SLEEPING SICKNESS SERVICE.						
Year	New surveys. Population examined by Teams.	Cases	Infection rate per cent.	Re-surveys. Population examined by Teams.	Cases.	Infection rate per cent.	Dispensary Cases.	Gaol Medical Cases.
1931	1,01,500	5 104	5 0	—	—	—	—	2,40
1932	145 671	13,29	9 10	4 1	642	15 5	—	2,77
1933	2,1	25,1	11	19,314	2,797	14 45	—	4,135
1934	369,233	41 458	11	1,2439	2 351	19 5	—	3,644
1935	407,503	54 364	19 5	—	—	—	16,454	4,072
1936	410 49	4 049	11 4	7 19	522	6 9	8 147	4,407
1937	437 70	55 758	6 8	9 651	53	6	8 661	4,522
1938	503,811	19 037	9 1	109 495	2,036	1 1	8 035	4,533
1939	383 40	1s 109	6 9	220 824	1 946	0 9	8 3,4	4,511
1940	191 099	1 89	9-28	93 861	18 9	1	8 843	4,617
1941	293 43	3,90s	1 6	36 207	1 065	1	8 811	1,942
1942	159 65	2,34	1 3	93 743	1 855	2 1	11,513	4,394
1943	19 616	4 4	1	45 78	5 437	3	13,104	1,34
Total	3,148,059	306,222	9-7	813 718	18,751	2	91,794	51,674

teams was partly due of course to the increased work being done and to the discovery of new areas. However, there was a genuine spread of the disease during those years. The increase in the general infection rate and in the numbers of cases treated at general medical stations confirm this. Most resurveys were done in what formerly were the worst areas. The comparatively low infection rates found recently are an indication of the progress made. In many areas which used to have infection rates of 20 per cent. and over, the present rates are 1·0 per cent or less. In such districts full resurveys are not being done sufficient information being obtained from spot surveys of some of the worst villages. Full resurveys have been carried out mainly in places where infection has been kept going by mining activities. As a result the average infection rates shown in the table do not give a true picture. They are higher than they would be if all districts had been given equal attention.

Details are given in the 1931 to 1942 Annual Reports on the Medical Services, Nigeria. In sleeping sickness areas mainly in the extensive central belt, a total of 3,148 069 people have been examined and 306,322 cases found, an infection rate of 9 7 per cent. In the worst parts of these areas 913,718 re-examinations have been made with an average infection rate of 2 2 per cent. In the same period 80,704 cases were treated at dispensaries and 43,674 at general hospitals giving a grand total of 450 451 Of these about 400,000 were new infections, the remainder relapsed cases.

During the years 1931–1935 the disease was still increasing. The average infection rate for new surveys was 13·6 per cent. that for resurveys 13 3 per cent. In the next period 1936–1940 the great mass of treatment done by the teams was having its effect, though there were considerable areas of heavy infection which had not been surveyed before. The average infection rate of these new areas was 8 5 per cent that found at resurveys was 1·4 per cent. In the last period, 1940–1943 most of the new areas were only lightly infected, the average infection rate being 1·6 per cent. while that for resurveys was 2·5 per cent. The higher figure for resurveys was largely due to most of the work having been done in areas badly affected by mining

DISPENSARIES

The first two trial sleeping sickness dispensaries were built in 1934 They were sufficiently successful for the number to be increased in subsequent years. The original intention was that they should be centres for the treatment of sleeping sickness, some simple general medical work being done to increase their popularity Gradually the scope and amount of general medical work was increased until it was necessary to post two dispensary attendants to many of them. At the start all attendants had had ample experience of sleeping sickness work with the teams though their knowledge of general medicine was very limited. To get over this they were posted for varying periods to

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local African hospitals. It was soon realized that the teaching at a busy African hospital was much too sporadic to be of much value. The knowledge such attendants did pick up was often hardly applicable to field dispensary practice.

A school for sleeping sickness dispensary attendants was started at Zaria. The course was a year and was divided between general medicine and simple training in health work. Again it was found that there was a tendency to put too much emphasis on hospital practice the health training too was based largely on town methods. Much of it had little bearing on the simpler methods of sanitation, etc., needed in cleaning up rural areas. It was decided to move the training school to Anchau, where it could be based on the local sleeping sickness dispensary The health training there could be done in conjunction with work in the neighbouring model settlement, a rural area as opposed to a large town.

The Sleeping Sickness Service policy is to try to make each dispensary a rural health centre. One attendant should take the sick parade, the other being responsible for health work. Both should tour as much as possible to carry out propaganda in outlying villages Model compounds are provided for them. The intention was to post a medical officer to each chain of dispensaries. He was to spend several days at a time at every dispensary in turn. While there he was to take the sick parade himself in order that each visit should serve as a short refresher course for the staff. In addition to supervising the local health work, he would be able to carry out small spot reserveys and to call in old patients for re-examination. Shortage of staff due to war conditions interfered with this programme. Inspections have had to be more haphazard than is desirable By the end of 1943 there were forty three sleeping sickness dispensaries and dressing stations, and nine more were under construction. Dispensary attendants trained in sleeping sickness work were posted to twenty nine of the original native administration dispensaries.

Last year about 75 000 general cases were treated at sleeping sickness dispensaries in addition to sleeping sickness patients. The table shows that the number of sleeping sickness cases has increased slightly in the last year or two Actually with the general decrease in the incidence of the disease there has been a considerable reduction in the annual number of cases treated at most of the dispensaries. Whereas nearly 5 000 cases a year used to be treated at the nine Zaria dispensaries, now only about 1,800 a year are treated at the full thirteen of them. In the country as a whole this decrease per dispensary has been more than counterbalanced by the increasing number of centres.

CONTROL OF MINES LABOUR.

Owing to the part tin mining was playing in spreading sleeping sickness in the districts to the south-west of the Plateau, a system of rigid control of mines labour had to be instituted in 1935 The trouble arose through alluvial mining

along stream beds heavily infested with tsetse. The nature of the country was such that wholesale clearings of the streams was impracticable, yet at one time 30 to 50 per cent of the labour had sleeping sickness The numbers of infected labourers working in close contact with tsetse-fly kept up a high rate of infection in the tsetse New labour, whether permanent tin miners from other parts of the country or local pagans working for a week or two as casual labourers, soon became infected. After varying periods such people would return to their homes, taking their infection with them. In this way a heavy incidence was kept up in the immediate locality in spite of every effort to reduce it by treatment campaigns. Similarly sleeping sickness must have been carried to more distant parts of the country, particularly when new gold areas were opened up and numbers of tin labourers went to work there.

The restrictions made it illegal for any labourer to work in the area without a permit. Before being engaged he has to submit to medical examination and be found free from sleeping sickness He had to agree to remain at work for at least 6 weeks, and to come for re-examination before being discharged. As well the whole labour force is examined for sleeping sickness every 6 weeks.

The effect of control was rapid whereas at the beginning it was found that 8 per cent of the labour contracted sleeping sickness in the interval between examinations, this figure soon fell to 0 5 to 1·0 per cent. The effect on the infection in the neighbouring villages from which most of the casual pagan labour had been drawn was just as striking By 1934 the average infection rate had risen to 14 3 per cent. in spite of much treatment. A year after the start of control it had fallen to 3 1 per cent. and later to less than 1 per cent.

In 1940 the system was extended to the neighbouring tin field in Jema a and to the Niger and Kabba-Ilorin gold fields Three sanitary superintendents were posted to supervise the examination and control of mines labour In the Kabba Ilorin mining area the results were particularly dramatic This gold field had only been opened about 2 years in an area where previously there had been a very low infection with a mild type of sleeping sickness. The influx of labourers from further north brought a more virulent type of disease which soon spread to epidemic proportions in the mining camps. There was considerable danger of the more severe form of the disease spreading throughout the local population. At the start of control the infection rate in the mining camps was in the neighbourhood of 35 per cent. It fell rapidly and now averages only 0 4 per cent The danger to the local population has been removed.

There has been considerable improvement in the Jema a tin areas as well though this has been less rapid than it should through many of the pagan labourers evading the restrictions In view of the war time drive for more tin, the strict enforcement of this control is not popular with the mining community Once the sleeping sickness staff could show that the infection rate among labourers who had missed re-examination was two or three times as high as that of the general population, there was less difficulty

Control in the Niger gold field is less easy because of the number of small camps scattered over a large area. The general incidence has been reduced considerably though it is still difficult to get the average figure below 2·0 per cent. A recently discovered gold field in the Southern Provinces is being kept under observation. No restrictions have been applied, as the regular examination of labour has shown that the average infection rate is not more than 1 per cent, and there have been no signs of infection in the local population.

At present the total labour force under strict control averages about 8,000. In 1943 an average of 1·4 per cent. of them were found to have contracted sleeping sickness every 6 weeks. If these cases had not been diagnosed and treated but had been allowed to infect more and more tsetse-fly while at work, the spread of infection would have been rapid. In quite a short time infection rates of 20 to 40 per cent. would have been general again.

Restrictions are particularly irksome to local pagan labour who like to work for a week or two to get a little money but refuse to leave their farms for 6 weeks at a time. To apply them satisfactorily needs a considerable staff. It would be much simpler if all labourers could be given prophylactic injections. If Bayer 205, or antrypol, were effective for a full 3 months, it would only be necessary to repeat these prophylactic injections every quarter. Labourers then would be able to leave any time they liked.

In 1936 numbers of healthy labourers were given prophylactic doses of 1 gramme of Bayer 205; others remained untreated. The results were promising though not conclusive. The danger of collapse after such injections led to the method being abandoned for the time being. Cases of collapse and even of death have occurred with the preliminary injection of 0·2 gramme of these drugs. There seems no justification for compelling a healthy labourer to take this risk. It is reported from the Belgian Congo that successful results are being obtained by the prophylactic injection of M & B. 800. This would appear to be a promising line of research. What is required is a compound which will protect for about 3 months and which can be administered without danger to healthy persons. It would be invaluable for such people as mines labourers whose occupation exposes them to an abnormal risk of sleeping sickness.

CLEARING.

The earlier work done at Shenfuri and Gadau showed that in much of Northern Nigeria limited amounts of stream clearing would protect against *Glossina tachinoides*. Recent work suggests that in the drier parts of the country protection against *G. palpalis* may be easier than had been thought previously. Details of the clearing technique are given by Nash (1937 and 1940). He divides clearing into two categories, aggressive, which aims at the eradication of tsetse fly from a good sized area of country and defensive, which is designed to protect the population from attack by tsetse during the course of their normal occupation.

The early clearing experiments at Shenfuri showed that by cutting out all thicket and low shade along a stream conditions became unsuitable for tsetse fly during the latter part of the dry season. Unfortunately during the rains tsetse-fly could utilize the high shade left and so would spread back into the clearings. Nash improved on this partial clearing and prevented tsetse-fly reoccupying the stream during the rains by putting in mile long barrier clearings. In these clearing has to be complete with the exception of grass. All shade has to be removed ruthlessly. In this way the partially cleared upper reaches of a stream system can be kept free from tsetse-fly. The number of high shade trees that can be spared varies with latitude and local conditions. The object is to preserve as many of the good trees as is compatible with letting in sufficient light and wind to make the locality unsuitable for tsetse fly during the hottest time of the year. Such methods could not be applicable to G. submorsitans unless large block clearings were put in to protect the area against wet season spread. The mile long barrier clearings along the streams would be ineffective.

While aggressive clearing is the method used in the Zaria settlement scheme which is described later defensive or protective clearing is in much more general use. It has long been recognized that a high incidence of sleeping sickness depends upon the close contact between man and tsetse fly. Taylor (1930) observed that in the Ganawuri villages tsetse were very rare, the few there lived practically in the villages and depended entirely on man and goats for their food. More than 50 per cent of the population had sleeping sickness. Since then other workers have noticed that heavy infection rates are not infrequent when tsetse-fly is very rare always provided that the fly are very near or actually inside the villages. The writer believes that the high infection rates which were to be found in much of Northern Nigeria were dependent upon tsetse-fly spreading along the streams right into the villages during part of the rains.

This is the basis of the protective campaigns which have been carried out in recent years. The object is to drive back the tsetse fly from immediate contact with human habitations and watering places and to protect the fords in the more important paths and trade routes. The amount of clearing required is too small to cause any serious deforestation. Providing the banks of the cleared stream are not farmed, the growth of grass prevents erosion.

At one time a number of administrative officers were given a special training in protective clearing. It was hoped that in the course of their normal touring they would be able to organize small clearing campaigns. Sleeping sickness medical officers gave what help they could while doing surveys and mass treatment. It was largely to obtain special staff to allow of these protective campaigns being put on a proper basis that application was made for assistance from the Colonial Development Fund to form a control side of the service.

The new staff started work in 1935. The control officer first tours a district to map the streams and villages. With the aid of the detailed findings of the

sleeping sickness survey he is able to plan the clearing required. The population figures indicate how many labourers will be available for each clearing. The actual work is then done by communal labour supervised by trained headmen working under his direction. It is rarely necessary for the adult male inhabitants of any village to do more than 2 or 3 days work to protect themselves. In succeeding years the regrowth in the clearings has to be slashed back. In time the amount of maintenance required decreases.

Up to the present communal clearing campaigns in Katsina and Zaria Provinces have safeguarded about 240 000 people from any serious risk of contracting sleeping sickness. Morris (1943) reported at the West African Tsetse Conference that in the northern parts of the Gold Coast quite limited clearing near an inhabited area had greatly reduced the incidence of *G palpalis*. He concluded that in the absence of game the tsetse fly were largely dependent on man for their food. On this source of supply being cut off the fly population tended to die out. Nash has made a similar observation in a village in Zaria Province. The village was in the centre of an isolated patch of high forest swarming with *G palpalis*. There was no game and few reptiles. Once the village was moved away from the patch of the forest the tsetse population died out. These findings suggest that in thickly populated areas where communal clearing campaigns have been carried out, the absence or great scarcity of game may cause the very limited amount of clearing done to have a greatly increased effect. The breaking of the contact between the fly and man, his chief food supply may cause a general diminution in the whole tsetse population.

Much of Nigeria is thickly populated and game is scanty both *G tachinoides* and *G palpalis* have been driven to depend upon man and his domestic animals for a large part of their food. Any wholesale destruction of the remaining game would make these tsetse still more dependent upon man and would increase the risk of sleeping sickness. On the other hand there are sporadic patches of *G submorsitans* in much of the less densely populated country. Any general increase in game would undoubtedly lead to a corresponding increase in this species. For this reason the policy of the Sleeping Sickness Service towards game has had to be one of *laissez faire*.

SLEEPING SICKNESS SETTLEMENTS

The removal of population as a means of protection against sleeping sickness has been widespread in Tanganyika Uganda, the Southern Sudan and the Belgian Congo The object was to break the contact between man and tsetse fly This can be done by moving people to tsetse free localities, or where suitable tsetse free country is not available, by establishing them sufficiently close together to make them safe from attack. In both the Sudan and the Belgian Congo people were moved from their homes in the tsetse infested valleys and concentrated along roads built on the higher ground and so relatively free from tsetse-fly In both countries *G palpalis* was the species con-

cerned. In Tanganyika, as no convenient tsetse free country was available, people were concentrated into settlements which their ordinary farming activities would keep free from *G morsitans*

In the earlier years in Nigeria there were several movements of district administrative headquarters because of tsetse fly but the first planned movement of any size was that of the Ganawuri tribes (TAYLOR, 1930) About 4 000 people were moved from their tsetse infested hillside villages on to a neighbouring fly free plain. This measure together with repeated mass treatment, brought to an end an epidemic which had been causing wholesale depopulation SYMES work in Kenya (1937) using hand catching by fly boys to free isolated blocks of *G palpalis* bush, suggests that it might have been possible to catch out all the very scanty *G palpalis* population in the Ganawuri villages If this had been known at the time it might have been easier than the wholesale removal of the population.

With the exception of a narrow belt in the north which is too and for tsetse fly and the relatively small area of the plateau which is practically treeless all the river systems in Nigeria are infested with tsetse Streams are everywhere so numerous that it would be very rare to find a convenient fly free area to which population could be moved. Ganawuri was the exception. With a population of 21 000 000 the country as a whole is much more densely populated than the East Africa territories.

In the north, both *G tachinoides* and *G palpalis* inhabit the narrow strip of riverine forest along the streams, often only a few yards thick. As this is rarely interfered with by ordinary farming there may still be plenty of tsetse-fly and a very close fly man contact in thickly populated country It was clear that movement of population to tsetse free areas would be out of the question on any considerable scale. A simple concentration of population would be completely ineffective.

It was decided that people ought only to be moved when the damage done by the disease is serious enough to warrant such an extreme measure. The population would then have to be made sufficiently dense and sited in such a manner that new settlements could he kept fly free by communal labour

At surveys done in Zaria Province in 1933-1935 district infection rates of 20 to 40 per cent. were common There was ample evidence of the damage being done and the continuous loss of population. The problem seemed to be most acute in the eastern districts of the Province where in some hamlets up to 50 per cent. of the people were infected. It was decided to incorporate plans for settlement work in the proposals drawn up at the end of 1935 for a comprehensive scheme for the control of sleeping sickness. It was clear that any movement of population in a predominantly Muslim area would need much careful planning There would be opportunities for rural development which should not be missed.

The approval of the general control scheme and appointment of new staff

made possible a scheme for making a tsetse free corridor through Ilara, Anchau and Kudaru districts of Zaria Province, extending from the main north-south railway line south-eastwards to the light railway connecting Zaria with Jos. Some 70 000 people would be affected population from outlying hamlets being moved into a corridor 60 to 70 miles long with an average width of 10 miles.

Work started towards the end of 1937. To begin with, progress was slow owing to the necessity of training new staff, both European and African. An account of the first 3 years work is given by NASH (1941). The first essential was to make a detailed map of the whole area showing distribution of population and streams. It was naturally desirable to move as few people as possible. Even then it would not be easy to keep them in their village units and fit them in among the existing population.

Before any removal of population, much basic research had to be done. To start with, it was clear that more land was being farmed than had been generally believed. Detailed maps 12 inches to the mile were made of all the farmland of a number of the old villages. It was found that each family had under actual cultivation about 2·2 acres per head. Allowing for fallow it was decided that each family would need about 34 acres, or 4·3 acres per head.

Similarly it was necessary to make a study of the types of vegetation and the significance of certain vegetation communities. Trial plots were put in to determine the best use that could be made of the different types of land. Detailed vegetation maps had to be made of the areas available for settlement. It then became possible to fit the population to be moved in between existing villages. The new villages had to be planned so that there would always be sufficient manpower available to keep all streams clear and so free from tsetse fly by 1 or 2 days' communal work each year.

While all this preliminary work was being done the streams were cleared by paid labour gangs supervised by the control staff. Where possible the partial clearing method was used, adequate barrier clearings being put in to prevent any danger of wet season infestations.

The building of new villages was planned and supervised by an administrative officer posted to the scheme for that purpose. Model compounds were built either singly or in blocks of two or three round an open well space at least 100 yards square. A fire break of about 100 feet was left between each block.

An extensive well sinking programme was carried out by a Foreman Water Supplies, seconded from the Geological Surveys. Wells were cement lined and provided with high collars to prevent contamination. Pit latrines were sunk in all compounds.

To start with, the scheme was viewed as one primarily designed to eradicate sleeping sickness. Gradually increasing emphasis has been placed on rural planning and development. Particular attention has been paid to hygiene, the encouragement of local industries, the introduction of new crops and fruit

trees, the encouragement of mixed farming and improved farming methods and the conservation of forests. Much work on these lines has been done with the co-operation of the Administration, Agricultural, Veterinary, Forestry and Geological Surveys departments.

Space will not permit of all these activities being described. The improvement of livestock alone has meant the establishment of a stock farm and the breeding of cattle for eventual distribution to villages as small communal herds. A pig industry has been established in the villages by the distribution of suitable European stock. This is becoming increasingly popular and profitable. Local poultry is being improved both by selective breeding and the introduction of Rhode Island Red stock. Donkeys have been introduced to some villages formerly without them. A Dan Bahar donkey stallion was purchased to improve the local breed. Two first-class stallion ponies are being kept to improve the local breed of horses. A veterinary clinic and animal inoculation camp help to control animal disease.

So far about 480 square miles of the corridor have been rendered fly free. About 5,000 people had to be moved. The scheme has come to be regarded as a model of rural development. The information obtained will be used increasingly for other rural development schemes which are to be sponsored by provincial welfare committees in different parts of the country.

In order that this should have a more direct influence on the rest of the province, the native administration and technical departments have co-operated in forming a provincial propaganda team for work in neighbouring districts. The team is being financed by the native administration. Each department has posted an experienced African member of its staff who teaches departmental policy under the general supervision of the medical propaganda officer a member of the control service. The leader of the unit is a representative of the Emir of Zaria. After a preliminary period of training in the settlement area the team has started work outside. This teaching should supplement the constant propaganda in both new and old villages in the settlement area.

PRESENT POSITION

The yearly figures for surveys of new areas and resurveys of old ones, given in the table, speak for themselves. The table also shows that about 0.5 per cent. of the population of the main infected areas are now being treated voluntarily each year at sleeping sickness dispensaries and general hospitals. Space will not permit of a detailed analysis district by district. In much of Zaria, Benue and Niger Provinces, where the original incidence of the disease was 15 to 25 per cent. present infection rates average about 1.0 to 1.5 per cent. Taking the Northern Provinces as a whole it is doubtful whether there is much more than a tenth of the old amount of the disease.

What this means in the way of progress is shown by the figures for Zaria Emirate. During the years 1923–1933 there was a severe epidemic throughout most of the Emirate. According to the native administration census figures

there was a 12 per cent. decrease in the number of adult male taxpayers in the 10-year period. By 1933 the total population had fallen to 373,195 people. That year the Sleeping Sickness Survey of the whole Emirate, district by district, was started Altogether 78,000 cases were diagnosed and treated. The average infection rate was 20·0 per cent.

From an investigation of vital statistics made in one of the central districts, HARDING (1940) proved that there was a direct correlation between death-rate and incidence of sleeping sickness. In a series of villages with an infection rate of 18·6 per cent. the death rate was 71·4 per thousand in a further series of villages with a 28·6 per cent. infection rate the death-rate was 104 per thousand. The average infection rate for the whole district was 23·2 per cent. and the average mortality was 84·2 per 1,000 The birth-rate was 64·7 per 1,000 There was clear evidence of depopulation due to sleeping sickness

With the reduction in the amount of the disease resulting from treatment, the establishment of dispensaries and protective measures, there was a corresponding change for the better in the population's figures. From 373,195 in 1933 the population increased to 402,257 in 1937 to 418,032 in 1939 and to 464,954 in 1943

In some areas there was no evidence that sleeping sickness was causing any immediate loss in population. It is certainly not claimed that the results of treatment were as strikingly valuable in such areas, though even there the improvement in general health should have had a beneficial effect on the energy and fertility of the population.

The position is much more satisfactory than it was 10 years ago. The spread has been stopped and, in most of the country the disease is under reasonable control. On the other hand, the fact that 21,000 cases were treated in 1943 shows that constant vigilance is necessary if even this relative improvement is to be maintained.

In 1942–43 a new area was discovered in a distant part of Bauchi Province, through native administration complaints of numerous deaths. These were found to be occurring in one town with a population of 1,314 About 23 per cent. of the whole town had sleeping sickness. Fortunately the survey was done before the disease had spread to any extent in the surrounding country Mass treatment and the clearing of tsetse foci in and near the town brought this small localized epidemic to an end. The incident is quoted to emphasize this need for vigilance.

When the staff position permits a new survey must be done in part of Ogoja Province. Fairly extensive resurveys will be needed in several districts of Zaria Province in two or three districts of Kano Province and in the Abua area of the Eastern Provinces.

A West African Tsetse Conference, consisting of representatives from French West and Equatorial Africa, the Belgian Congo Liberia, as well as from various British West African territories, was held at Lagos in July 1943. The discussions emphasized the differences in sleeping sickness in different

localities. The great diversity in behaviour of various strains and in the resistance of different tribes to them, make it impossible to lay down any standard treatment for all territories. The need for more research work was brought out, particularly with a view to investigating the causes of these differences in strains and in resistance. Arrangements were made for a closer co-ordination of all sleeping sickness work in the various countries.

In recent years most research on trypanosomiasis in West Africa had to be suspended owing to the need for devoting all resources of staff and funds to treatment and control. It has become clear recently that essential research will have to be resumed if further progress is to become possible. Proposals have been framed for the establishment of a Central Trypanosomiasis Research Institute to be established at Kaduna Northern Nigeria. Such an institute would serve all British West Africa and would be available for foreign territories if required. A request is to be made for assistance from the Colonial Development Fund.

The writer's thanks are due to all past and present members of the Sleeping Sickness Service, both Europeans and Africans, as it is they who carried out the work described in this paper.

SUMMARY

Three types of sleeping sickness are described as occurring in Nigeria— the mild type, the toxic and the nervous. The great majority of the cases found at sleeping sickness surveys belong to the first group. Patients suffer from occasional attacks of headache and fever and from some weakness very little else. It is their increased susceptibility to intercurrent diseases which often caused the depopulation found in some of the more heavily infected areas. In the second group which is much more rare toxaemia is the salient feature. In the third there are signs of progressive nervous involvement. The proportion of patients suffering from the three types varies. Even in the more virulent epidemics the mild form is common.

In Northern Nigeria there is a striking correlation between the areas of infection and the lines of communication, railways roads and mining areas. The main zone is confined to the central part of the country. Peripheral areas, though heavily infested with tsetse-fly are still practically free from infection.

Nigerian policy is to establish convenient permanent treatment centres once a full survey of the whole population has been followed by mass treatment. The information obtained at the survey makes the planning of effective protective measures possible. The work of the sleeping sickness teams, dispensaries and the control of mines labour is described.

From 1931 to 1943 a total of 3 148 069 people were examined in new areas and 306 322 cases found, an infection rate of 9 7 per cent. In the worst areas 913 718 people were re-examined and an infection rate of 2 2 per cent. found. During the first 5 years of this period the disease was still increasing. The

average infection rate was 13·6 per cent. In the next 5 years the spread had been stopped though the infection rate was still high in the remaining new areas. From 1941 onwards the new areas discovered had a low infection rate. The rate for resurveys of what were formerly some of the worst areas was 2·5 per cent. Taking Northern Nigeria as a whole, it is doubtful if there is much more than a tenth of the old amount of infection.

The sleeping sickness dispensary system has been expanded during the last few years. Some 80 704 cases of sleeping sickness have been treated. Practically all were voluntary attendances. The general medical and health work also has been improved.

With the 43,674 cases treated at general medical stations, a total of 450,451 cases have been treated in the last 13 years. Of these about 400,000 were new cases, the remainder relapses.

The control of the disease by communal clearing campaigns is described. So far about 240 400 people have been protected from any serious risk of contracting sleeping sickness. A brief account is also given of the Zaria sleeping sickness settlement scheme. So much attention has been paid to all aspects of rural planning and development in the settlement area that it has come to be regarded as a model of rural development.

The changes in population figures, particularly in Zaria Emirate, give a striking indication of what has been accomplished. In the period 1923-1933, when the disease was increasing the population fell by about 12 per cent. At the survey of the whole Emirate 78,000 cases were diagnosed and treated, an infection rate of 20 per cent. There was direct evidence of correlation between infection rates and mortality. With the decrease in sleeping sickness consequent on treatment and control, depopulation stopped. Since 1933 the total population has increased about 24 per cent. It is not claimed that results of treatment have been as valuable in the milder area where there was no sign of the disease causing immediate loss of population.

The general position is much more satisfactory throughout the country though constant vigilance is necessary if this relative improvement is to be maintained.

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VARIOLA MINOR IN KENYA.

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INTRODUCTION

The observations recorded were made during a period when the author was Assistant Medical Officer of Health and Assistant Port Health Officer, Mombasa, and later while Medical Officer at Kerugoya Hospital. This hospital is situated in a native reserve in the Central Province of Kenya.

The first batch of cases of variola minor, twenty one in all, was observed in Mombasa, between 9th February and 13th March, 1943. The second batch, observed at Kerugoya, occurred between 13th June and 12th August, 1943. A total of forty-two cases were seen during this latter period.

MOMBASA CASES

Of the twenty-one Mombasa cases, eighteen were imported. All eighteen of these were Arabs who either arrived having the fully developed picture or who, on arrival, were in the incubation period of the disease. All had travelled by dhow direct from Arabia. Seven separate dhows were involved, and as the only common port of call was Mukulla, it was inferred that the infection originated from there or from the neighbouring district.

Of the eighteen imported cases, five developed the disease under routine surveillance to which it was customary to subject all foreign dhows in addition to dhows from those ports whose sanitary condition was unknown, and, need less to say, from declared infected ports. In point of fact, the first two cases seen were discovered during this routine surveillance. Of the further cases, seen four arrived at Mombasa in the eruptive stage, and nine developed the disease while under observation in the Infectious Diseases Hospital.

The final three cases occurred in the indigenous population of Mombasa. All of these were African sweepers living in the Municipal Labour Lines which were situated next to the Infectious Diseases Hospital. These three cases were discovered between 9th and 13th March. No further cases were notified up to 8th April, when the author was transferred to Kerugoya. The position during this period was aggravated by the fact that owing to food distribution

difficulties in the town, intensive efforts were being made by the administration to send all unemployed natives, and those not in essential work, back to their reserves up-country. Many hundreds were leaving Mombasa daily and it is therefore not unlikely that, in spite of rigorous vaccination and surveillance of all contacts, one or more of them travelled up-country while incubating the disease.

It is speculative to consider the mode of infection from the Arabs to the three municipal employees, but it would appear extremely likely that this occurred between the hospital and the Municipal Lines. again, it appeared more likely from the evidence, in spite of vigorous denials on both sides, that the infection was conveyed by direct contact rather than by aerial convection.

Measures undertaken for control of the disease

It is not intended in this paper to examine in detail the public health aspects of the disease, or the measures taken to combat the spread of infection. Regarding the latter, it is sufficient to say that the disease was treated as though it were variola major. Vaccination and surveillance of all known contacts were the chief weapons relied upon, both when the infection was isolated to the Arabs from the dhows, and also when it had spread to the Municipal Lines. In a small area round the Municipal Lines and Infectious Diseases Hospital, total compulsory vaccination of all people was undertaken. all Africans travelling up-country were vaccinated at the railway station. In all, during the period 13th March to 7th April, about 2,600 people were vaccinated. No mass vaccination of Mombasa Municipality was undertaken.

KERUGOYA CASES.

In the Kerugoya series, forty two cases were discovered during the period 13th June to 12th August. The first case was discovered on 13th June, mass vaccination of the whole district was started on 17th June, and was completed on 12th August, 8 weeks later. A total of 166,151 people were vaccinated during this time and an inhabited area of roughly 1,220 square miles was covered. All the vaccination was done by three teams of African health workers and dressers, on occasion supplemented by a fourth team. Although the figure 166,151 surpassed the estimated census of 1941 by 11,500, it was considered that probably only about 90 to 95 per cent. of the actual population had been vaccinated. This was brought out by the fact that during the period 13th August to 2nd October four further cases of variola minor, all occurring in unvaccinated persons, were admitted to hospital.

CLINICAL ASPECTS OF VARIOLA MINOR.

Incubation period

It was impossible to estimate the incubation period of the disease in the cases under review, as no adequate history of contact could be elicited from

any of the cases. Taking into consideration the primitive people involved this is really not surprising. In addition such close enquiries as were made possibly entertained a degree of suspicion in the minds of the patients that something unpleasant might happen to the individuals from whom they had contracted the disease.

In the Mombasa series, eighteen of the cases were imported, having contracted the infection outside Kenya, and there was no means of telling the actual date of infection. The three remaining cases occurring in the indigenous population had some reason for not admitting contact, if in fact, the infection had been gained by nefarious visits to the compound of the Infectious Diseases Hospital. In the Kerugoya cases, owing to lack of space, few contacts could be isolated for observation and, due to the extensive area to be covered with a relatively small staff routine surveillance of contacts was impossible. Of the few contacts isolated for observation none developed smallpox. Presumably all had been aborted by vaccination in the early incubation period of the disease.

From the point of view of preventive measures the incubation period was taken to be 14 days from infection to the appearance of the rash. MANSON-BAHR (1940) states that the incubation period averages about 14 days. PRICE (1937) states that the incubation period is 10 to 15 days or longer.

Symptoms

The great majority of cases were not seen until the rash had erupted but almost all of them gave a history of fever headache and malaise for a period of 3 or 4 days before the rash appeared. Table I gives an indication of the degree of pyrexia obtaining in the eight cases which were observed in the prodromal period.

In nearly all cases the temperature dropped to normal on or soon after the appearance of the rash and usually remained normal. Secondary suppurative fever was observed in sixteen cases in the whole series. This fever

TABLE I

Highest temperature in F reached in pro-dromal period	98·6	100·2	102·2	103	104
Number of cases	1	2	2	2	1

varied, but usually continued for a period of 3 to 4 days, ranging between 100 and 102 F.

The temperature charts of two cases exhibiting secondary suppurative

fever are illustrated. In the first case (Chart 1), the rash passed through the vesicular and pustular stages fairly rapidly without extensive suppuration, and the temperature was mild during the pustular stage lasting for a period of 3 days. The chart, in addition illustrates the prodromal pyrexia and drop of temperature with the appearance of the rash.

In the other case (Chart 2) it can be seen that the vesicular stage was of about the same duration as in Chart 1 but the pustular stage was more drawn out, lasting for a period of 7 days. The fever was more pronounced and, in fact, clinically the child was ill and toxic.

The Rash.—In the cases observed during the prodromal period, and from case histories of others, it would seem that in the majority of instances the
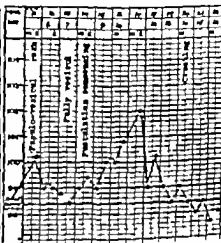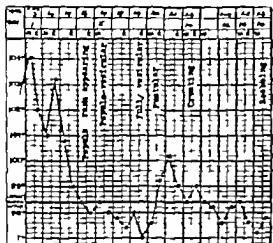
CHART 1   S.B H. adult male   CHART 2. B.B. boy aged about 11

TEMPERATURE CHARTS—CASES OF VARIOLA MINOR.

rash erupts on the 3rd or 4th day of the illness, appearing as a papular eruption which can be felt more easily than seen, especially in dark skins. This rapidly becomes papulo-vesicular in about 24 hours, and fully vesicular after 2 or 3 days. In the vesicular stage umbilication of the lesions is marked, and the individual lesion is not easily collapsed on pricking it. The rash does not appear in crops, but new lesions may appear during the first 24 hours following the initial papular eruption. By the time, however that vesiculation is advanced the whole rash appears to be in the same stage. Following vesiculation, pustulation sets in, with or without constitutional symptoms after the onset of pustulation the individual pock begins to lose its owing to the destruction of the septa binding down its tough covering pustulation. crusting occurs at a variable interval which may from 2 to 6 days. With the onset of this the lesions dry and are a thick yellowish crust, which, in time takes on the appearance

The average period from the eruption of the rash to the appearance of crusting was found to be about 10 days but varied between 7 and 14 days. Finally, on separation of the scab a central pink area is observed. This is depressed below the level of the surrounding skin, and is itself surrounded by a halo of deeply pigmented skin which in most cases was quite black. In time the central area becomes hyperpigmented and the final lesion has the appearance of a round black blob, averaging about half a centimetre in diameter and with a slight but definite central depression. In no case was the rash confluent.

An important feature of the rash, especially when the differential diagnosis with chicken pox is being entertained, is the depth of the lesion in the skin. The pock in variola minor can be seen and felt to be situated deeply in the skin, unlike the lesion in chicken-pox which lies superficially.

Distribution of the rash.—The distribution of the rash, as in variola major, is typically centrifugal, being more dense on the limbs and face than on the body. On the limbs in the majority of cases the rash is characteristically evident on the palms of the hands and on the soles of the feet, and when occurring in the latter position occasions considerable pain while walking. On the arms it is denser on the extensor rather than on the flexor aspects. On the chest and abdomen the rash is sparse but is more in evidence on the back, and particularly on the buttocks. On the face the eruption is well in evidence, being distributed chiefly on the forehead, cheeks and chin. A few lesions are usually scattered over the occipital area. The eruption has a marked tendency to accumulate round points of irritation such as ulcers, the trouser belt area buttocks, and old scars and to avoid protected areas such as the armpits, the groins, and underneath the female breast. In only two cases of the series were the mucous membranes affected to any degree, and in both cases the conjunctivae, and nasal and oral mucous membranes were extensively involved. The rash cleared normally from these places and there were no untoward complications.

VARIOLA MINOR MODIFIED BY VACCINATION

In observations on variola major PRICE (1937) quoting RICKETS, states

The period of incubation of smallpox, counting to the outcrop of the rash may be taken as 14 days. If this period be divided into three intervals comprising 7 days, 3 days and 4 days, it will be accurate in the main, to say that a successful vaccination done in the first period will wholly prevent the attack, done in the second will have more or less effect in modifying the eruption, and done in the third will merely add to the patient's troubles.

In the present series, owing to the mildness of constitutional symptoms in most cases, and the mildness of the eruption even in some unvaccinated cases it was difficult to assess to what extent the disease had been modified in those persons vaccinated in the incubation period.

There were in all fifteen cases vaccinated in the incubation and prodromal

periods who subsequently developed variola minor Table II shows the period in days before the eruption of the rash on which the vaccination was performed, and indicates in which cases the disease was modified and in which it was unmodified this is compared with the periods in variola major in which vaccination is said to abort, modify or have no influence on the disease.

Owing to the small number of cases no hard and fast conclusions can be made, but the inference can be drawn that if vaccination is performed between 4 and 5 days immediately preceding the eruption, the disease will be unmodified, between the 6th and 9th day the disease stands about an even chance of being modified, and previous to the 9th day before the appearance of the rash or the 5th day in the incubation period, it can be said with more certainty that the disease will be aborted. Many contacts, especially in the Kerugoya reserve, who did not contract the disease were vaccinated in this latter period unfortunately no numbers were kept, but these must have been quite considerable.

Clinical appearance of modified variola minor

In these cases constitutional symptoms are extremely slight if present at all, the rash is sparse or very sparse, and there may be only one or two lesions present. The eruption has the typical appearance of that in the unmodified cases and when present to any degree the distribution can be seen to be the same. The patients in addition will be found to have marks of recent vaccina-

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DESCRIPTION OF PLATE.

Variola Minor

Fig. 1—Illustrating the general appearance of the rash from the front The concentration of the rash can be seen chiefly on the face, thighs, round the knees and on the lower legs, the relative sparseness of the rash can be noted on the chest and abdomen, the flexor aspect of the left arm and in the groin. Umbilication can be observed in a few lesions on the chest, but is mostly absent as the rash is in the stage of pustulation

Fig. 3—The heavy concentration of the rash round the buttocks, and round the waist

The area of the trouser belt (points of irritation) can be observed. Note the concentration of the rash on the extensor aspects of the arms, and also the presence of few lesions in the occipital area

Mod fied Variola Minor

Fig. 5—The three successful accination marks can be seen on the upper left arm, a few pocks an be observed scattered on the upper arm, the face, and the trunk. The lesion on the trunk giv some idea of the depth of the lesion. This man contracted his rash seven days after accination. Constitutional symptoms were negligible

Fig. —Same case as in Fig 1 taken at the same time. By comparing ith figure 1 the relative higher concentration of the rash on the extensor aspect rather than the flexor aspect can be seen. The presence of the rash on the hands and between the fingers can be noted, umbilication of the rash can be observed in one or two of the lesions.

Fig. 4—The appearance of the rash on the face gives some idea of the depth of the lesions, hich at this stage (pustulation) appea s round pearls set into the skin

Fig. 6—The four successful vaccination marks can be seen on the left arm, two pocks can be observed on this arm, three on the right, and two on the trunk. This boy developed an extremely sparse rash eight days after accination.

TABLE II

Modification by vaccination of variola major	Unmodified					Modified					Aborted		
Number of days between vaccination and eruption of rash	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of cases vaccinated in above periods	—	1	1	2	1	4	3	1	2	—	—	—	—
Variola minor modified or not	—	UM	UM	UM	UM	UM2 M2	UM1 M*	M1	UM1 M1	—			

Unmodified.	60 Modified.	
	40 Unmodified	Aborted

M = Modified.   UM = Unmodified

tion or good old scars. Plate Figs. 5 and 6 illustrate the appearance of the rash in two such modified cases.

Complications.—In the present series the only complications observed were (1) the appearance of the rash on the conjunctivae nasal and buccal mucous membranes in two cases, and (2) a case complicated by pregnancy resulting in the birth of a live child having a variolous rash. This case is described later in this paper

Mortality.—In no case in the whole series of sixty three did a death occur MANSON BAHR (1940) quoting RIBAS and MOODY states that the mortality in variola minor is 0·45 per cent.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis with variola major is in some cases apt to be difficult. The appearance and distribution of the rash is identical in both diseases, with the exception that in variola major the lesions are inclined to lie deeper in the skin and all the stages of the rash, especially that of pustulation, are usually more drawn out. However in the early papular and vesicular stages when the diagnosis is more important, the high temperature, severe constitutional symptoms, and profuseness of the rash are the only indications that the case may be one of major smallpox, but it must be remembered that sometimes variola minor is ushered in with a high temperature and severe constitutional disturbance (Chart 1 p 448) Confluency of the rash would warrant a diagnosis of variola major but it is said that occasionally a case of variola minor may be confluent (MANSON BAHR, 1940) It might be more

difficult to differentiate between a case of variola major modified by vaccination and a case of variola minor and in the absence of other typical cases of either type might be almost impossible. The absolute diagnosis would then have to await the appearance of other cases. However seeing that from the public health point of view the tendency is to treat both diseases in the same way the differential diagnosis would appear to have a limited practical application. It is, however more reassuring to know from the start of an outbreak with which type of smallpox one is dealing

The differential diagnosis with varicella is more important, but fortunately rather easier. The distribution of the rash in chicken pox is typically centripetal, protected areas are involved, the lesion is more superficial in the skin, collapses easily when pricked with a sterile needle, is very seldom seen on the palms of the hands and soles of the feet, and cropping is a marked feature of the rash. These features, when contrasted with those of variola minor should lead to no difficulty in the majority of cases. Cases sometimes occur however, which may be extremely difficult, especially in the absence of an outbreak of variola minor. The differential diagnosis in these cases can be made in a previously unvaccinated individual, by vaccinating him, with controls if possible and observing whether the vaccination is successful or unsuccessful.

Generalized cutaneous vaccinia might cause some confusion in the diagnosis. In generalized vaccinia there will be evidence of recent vaccination. The eruption seldom appears *earlier* than 9 days after vaccination in this condition, whereas the smallpox eruption, either in a modified or unmodified form, will probably occur *within* the first 9 days after vaccination, as the complete abortion of the disease is probable if vaccination has been carried out within 5 days of exposure to infection. It should be borne in mind also that generalized vaccinia very seldom affects adults, and that the distribution of the rash is neither typically centrifugal nor centripetal.

In the series under review a case of secondary yaws was mistaken for variola minor in the crusting stage. The yaws lesions were all of about the same size namely ½-centimetre in diameter and capped with a yellow crust. The distribution and characters of the rash in this particular case had a very striking resemblance to that of the crusting stage in variola minor with the exception that the yaws lesions had a slightly more vivid yellow coloration. The history of a primary yaw the duration of the lesions, the demonstration of *S pertenue* in fluid obtained from the lesions, the ultimate progress and response to bismuth, should easily prevent confusion. In this particular case vaccination was performed successfully

The diagnosis of the papular stage of variola minor was entertained in two further cases of secondary syphilide, but, again, the duration, the typical distribution, and later the fact that the rash did not progress through the various stages of minor smallpox, put the cases out of court. Positive *Kahn* reaction in both patients was, subsequently, additional confirmation.

A bad case of secondarily infected scabies may superficially resemble a secondarily infected variola rash. The lesions, however are not so deep in the skin, any resemblance to the distribution of a smallpox rash is incidental and, finally, burrows can be found and the acarus identified from the lesions.

In doubtful and suspicious cases vaccination should always be done as a diagnostic test. The failure of a vaccination to take, performed with fresh lymph and good technique, in a previously unvaccinated subject with a suspicious rash, lends very heavy evidence to the fact that the rash is in fact, a manifestation of either variola major or minor. The disadvantage of the procedure is, however that the diagnosis is delayed for 5 or 6 days by the end of which time the diagnosis will, in all probability have become quite clear. In a few cases, however it will be found to be of diagnostic value, and for this reason alone the procedure is thought to be well worth practising in doubtful cases. In the present series of cases vaccination of all subjects admitted with a variola minor rash was practised, and in not one case was the vaccination successful. In many instances unvaccinated controls were done at the same time under the same conditions and with the same lymph, and all controls had successful takes.

VARIOLA MINOR CONTRACTED IN UTERO

A rare but interesting case of variola minor was observed among the cases at Kerugoya, this was the birth of an infant in hospital with the stigmata of the disease. No reference could be found, in such literature as was available, regarding this complication in variola minor. PRICE (1937) makes brief reference to the complication in variola major stating that abortion or premature delivery is to be expected in all severe attacks and that few of the children born in these circumstances survive, sometimes they actually show the rash or its scars at birth.

An adult female Mkikuyu woman, aged about 20 a primipara, was admitted to hospital in labour on 2nd September 1943. On examination she was found to have suffered from undoubted variola minor. Deeply pigmented pock marks with a typical distribution were noted, chiefly on the face and arms particularly the extensor surfaces and on the hands legs and feet, including the soles of the feet. The variolous rash had completely healed, leaving the dark pigmented areas. It was thought that the rash was of a duration of at least 1½ months. She showed no marks of previous vaccination and denied that she had ever been vaccinated. Further history elicited from her brought out that on 1st July when the mass vaccination was being carried on in her area, the rash had begun erupting. Two days previous to its appearance she stated that she had had fever. This would indicate that the disease occurred 2 months before admission to hospital.

Four hours after admission she had a normal and spontaneous labour and was delivered of a full-term female child, whose weight was 5½ lb. The

infant was found to have an almost healed smallpox rash. The distribution was typical, being chiefly on the face, arms, and legs, with only a few lesions on the body. The rash consisted of circular areas of deep pigmentation, with a surrounding area of hyperaemia. It was exactly similar to the healed rash of variola minor as seen in adults with the exception that the area of hyperaemia surrounding the pigmented areas was more marked, probably due to the lighter and more delicate skin of the infant. In addition a few pustules were present on the soles of the feet; these subsequently rapidly dried and scabbed. A Kahn test performed on the mother proved to be negative. Both the mother and child were vaccinated with fresh lymph on 10th September; they were discharged from hospital on the 13th, and to that date no signs of a "take" could be observed. Unfortunately they did not return to the hospital after a week for final observation of the vaccination result, but both were seen again on 16th November 2 months later when no signs were present in mother or child of the vaccination having taken. The rash on the mother had faded on the exposed portions of the body, namely on the forearms face and legs, and was dark brown, but on the areas of the body protected by clothes the rash was still black. Slight punctate pitting was observable in most lesions. The rash on the child had become depigmented, and consisted of small, markedly hypopigmented areas, which were becoming depressed, and it looked as if pitting was going to be of the order of that following variola major. It is difficult to explain why the rash in the infant lost its pigmentation and took on the appearance of a variola major scar. It can probably be explained by the fact that owing to the more delicate skin of the infant, the rash in utero was situated deeper in the skin than is normal in variola minor with the consequent destruction of more skin tissue.

METHOD OF VACCINATION ADOPTED AND VACCINIAL COMPLICATIONS ENCOUNTERED

Method of Vaccination

Having due regard to the fact that, in the Kerugoya reserve at least, the probability was remote of the native population being revaccinated again in a lifetime unless in the presence of an outbreak of smallpox, it was desirable that each person should receive the maximum immunity possible.

With this end in view and working on the statistical assumption that immunity to smallpox is directly proportionate to the number and area of old vaccination marks all persons with the exception of infants without teeth were vaccinated by the scarification method in four separate areas about 1 inch apart on one arm, each area consisting of four scratches about $\frac{1}{4}$ inch long. The resulting appearance was $||||$ $||||$. Infants were vaccinated with three such areas. In addition, it was impressed on the vaccinators the absolute necessity for keeping to a rigid technique, which was laid down, and for using fresh lymph. The results were extraordinarily good, and the percentage of unsuccessful "takes" must have been very low.

Many of the natives had never been vaccinated previously, and though no record was kept it was estimated that probably at a maximum, only about 10 per cent. of natives showed evidence of old vaccination marks. Thus probably about 90 per cent. of people were vaccinated for the first time, the primary vaccinations performed covering all age groups fairly evenly

The vaccinial ' take' observed in both Europeans and natives was of the usual order seen in primary vaccinations with a temperature from 100 to 101° F for 1 or 2 days at the end of the 1st and beginning of the 2nd week, accompanied by an axillary adenitis, headache and malaise.

Complications Following Vaccination

By far the commonest complication observed was the infection and suppuration of the vaccinial vesicle caused by scratching This was aggravated by the uncleanly habits of many of the natives In addition to this common complication, one case of generalized (cutaneous) vaccinia was observed, and one case of post vaccinial encephalitis. A case of acute syphilitic transverse myelitis occurring with sudden onset 12 days after vaccination in a male aged 30 who had been vaccinated for the first time in his life was at first thought to be a case of post vaccinial encephalitis Real doubts were first thrown on the diagnosis when the Kahn reaction was returned $+++$ and finally the report on sections of material obtained postmortem confirmed the diagnosis No other complications were observed, but it must be stressed that in such a large and, to a great extent, uneducated community spread over such an area, it is not unlikely that other and even fatal complications occurred, which were never brought to the notice of the author

Sepsis following Vaccination—Many arms became badly secondarily infected, chiefly due to scratching and one or two cases were seen with really bad and deep ulcers reaching down to underlying muscle This latter state of affairs is not surprising in view of the practice of certain natives of rubbing cow dung and earth into the arm lesion directly after vaccination This procedure, although leading to the formation of good scars cannot be said to have enhanced the chances of good immunity and thus it is likely that there are natives in the reserve showing good old vaccination scars who may have little or no immunity due to this undesirable practice.

GENERALIZED (CUTANEOUS) VACCINIA.

This condition would appear to be an extremely rare complication of vaccination.

JUBB (1943) quotes STEVENSON and COX who in a series of 3,289 733 vaccinations, found thirty four accepted cases of generalized vaccinia giving an incidence rate of 1 in 96 758 The fatality rate for this series was 11 7 per cent. SHELDON (1936) gives an incidence of one case in about 100 000 vaccinations.

Case History

The case under consideration occurred in a young Arab boy aged about 12, who was admitted to the Infectious Diseases Hospital Mombasa, on 30th December 1942. It will be noted that this was before the arrival of variola minor at Mombasa in the dhows from Mukulla. This boy arrived in Mombasa on 17th December in an Arab dhow which, having called at Mogadishu had left that port on 12th December. He had been vaccinated at Mogadishu on 10th December in company with the rest of the crew but there was no evidence of his vaccination having taken. The whole crew including the boy were revaccinated on the day of arrival at Mombasa, and sixteen of the crew, including the boy were held under surveillance as they did not have good old vaccination marks. All these people were seen daily. On 30th December 13 days after vaccination, the patient was found to have a fairly extensive papular rash, which in some parts was becoming vesicular. This was distributed chiefly on both arms, and on the left shoulder just above the vaccination marks, where it was most in evidence. Additional lesions, but not so many were scattered on the back, legs and abdomen in that order of density. The rash was situated superficially in the skin. The lesions varied in size to a certain extent, most being of about the size of an average varicella pock, but some were considerably larger especially those on the left arm, where one lesion was about half an inch across. There was no umbilication. No lesions were present on the chest, face or mucous membranes. He was, in addition, suffering from coryza and had herpes labialis. His temperature was 99 F., and he appeared generally quite fit. The vaccination performed on 17th December had taken well, and he had four large areas about ½ inch in diameter on the upper left arm. Secondary infection of these had occurred through scratching. The fact that this might be a rash due to auto-inoculation was borne in mind, but was ruled out as there were a few papulo-vesicles on a portion of his back, which he could not reach with either hand. Moreover the sites most frequent in auto-inoculation are the face and the front of the body J[czar] (1945) and it has been noted that no lesions occurred on the face or chest, and that relatively few were present on the abdomen. He was admitted to hospital for observation. On 31st December 1942, when his temperature was normal, the rash had become more vesicular and a few fresh papules had appeared on the trunk. Twenty-four hours later a very few more new papules had appeared on the trunk, and owing to the itchiness of the rash, some of the older vesicles had been scratched off. Treatment with 1 per cent. potassium permanganate applied twice daily to the lesions was sorted.

By the following day no further crops had appeared; but scabbing had started, he still had a slight coryza, and the herpes was still present. A week later nearly all the scabs had separated, the herpes and coryza had cleared, and no further crops had occurred. The temperature had remained normal during the whole course of the disease, with the exception of the 1st day.

He was discharged from hospital on 13th January, 1943 when all the scabs had separated with the exception of two of the primary vaccination scabs.

Meanwhile the crew of the dhow were under daily surveillance until 15th January 1943 when they were released. None of them developed any rash or had any temperature not accountable for by a normal vaccinial reaction. All had been vaccinated with the same batch of lymph, and of the sixteen who previously had had no evidence of recent "takes" nor good old marks had successful vaccination "takes."

Discussion

The differential diagnosis, it seemed, lay between the following four conditions —

   (1) Auto-inoculation. (2) Modified smallpox (either variola major or minor) (3) Chicken-pox occurring incidentally (4) Generalized vaccinia.

The question of auto-inoculation has been discussed, and on the grounds given can, in the author's opinion be ruled out.

The lesions, although having a distribution roughly similar to smallpox,

were not deep in the skin were not umbilicated, were not of a uniform size the face was not affected in addition, the rash came out in crops and first appeared 13 days after a successful vaccination Further, none of the crew under surveillance contracted smallpox.

There was the possibility that the child might have contracted chicken-pox as an incidental infection but the distribution was not that of a varicella rash some of the lesions particularly those on the arm were bigger than the pocks usually seen in chicken-pox and during the period of 29 days from 17th December to 15th January no other person on the dhow developed varicella

By a process of elimination the diagnosis of generalized vaccinia was arrived at This diagnosis received further support from observations on generalized vaccinia in a paper by JUBB (1943) who states that the following should be borne in mind when considering the diagnosis —

(1) Auto-inoculation should be excluded.

(2) The eruption does not appear earlier than the fourth and seldom earlier than the 9th day after vaccination

(3) The eruption must be elsewhere than in the neighbourhood of the vaccination site

(4) There must be a vesicular stage

Other relevant points contained in JUBB s paper are —

(5) The only case in the thirty four cases of generalized vaccinia investigated was beyond childhood and this patient was 17 years old

(6) No record exists at the Ministry of Health, of generalized vaccinia in secondary or tertiary vaccination although eight cases are quoted as having occurred in secondary and one case in a tertiary vaccination. The eight cases occurring after secondary vaccination had all been *successfully* vaccinated in earlier life it is not, however stated whether in the case of the tertiary vaccination, the two previous vaccinations had been *successful*

(7) By far the commonest period for the appearance of the rash after vaccination is from the 9th to the 14th day

(8) Apyrexia is not uncommon in generalized vaccinia

(9) The eruption does not ordinarily go beyond one crop but new crops may appear up to 5 or 6 weeks although this is unusual.

(10) The eruption usually affects the trunk more than the limbs, and may appear on the mucous membranes

All the first four essential points are satisfied in the case history described, but regarding the last six subsidiary points consistency is not quite so marked. The boy was aged about 12, which would probably be on the high side when compared with JUBB s cases, depending on exactly at what age the period of childhood can be said to terminate Point six is satisfied in that this was the first successful vaccination undergone by the patient he had undergone an unsuccessful attempt at vaccination 7 days previously Points seven and eight are both fulfilled There is some diversion when it comes to points nine and ten

Three crops of rash were noted in the case described appearing over a period of 3 days which is apparently an uncommon occurrence. As regards the final point, the eruption in this case was the reverse of the most common distribution, in that it was more marked on the limbs especially the upper limbs, than on the trunk but JEBB states later in his paper that the distribution is not specific, and the eruption may arise on any part of the skin.

POST VACCINAL ENCEPHALITIS.

Increasing notice has been taken of this condition since 1923 and, in view of its rare occurrence perhaps rather too much stress has been attached to this complication following vaccination, leading in some instances to rather over rated anxiety by medical practitioners. It should be pointed out, however that, in view of the facts, this complication is by no means a contra-indication to vaccination, but rather a definite pointer that primary vaccination should be undertaken in infancy and not at school age or later in life.

Case History

The patient, a male native child aged 10 was admitted to the Native Civil Hospital Mombasa, on 8th March, 1943 with urinary schistosomiasis. On 9th March, examination of the urine revealed *S. haematobium*, his blood slide was negative for malarial parasites, and macroscopic examination of his stools revealed *Ankylostoma* and *Strongyloides* ova. He was treated with ½ grain of tartar emetic every alternate day and was given eight injections up till 23rd March. He was, in addition, on 10th March, treated with oil of chenopodium for his ankylostomiasis. His urine, examined macroscopically on 22nd March, still showed eggs of *S. haematobium*

On 17th March he was vaccinated at company with 392 other patients and staff in the hospital, including about fifteen other children undergoing bilharzial treatment. This was thought essential as a case of variola minor had been admitted to the hospital in the pre-eruptive stage, and was diagnosed 24 hours later on the appearance of the rash. The same batch of lymph was used for all people This was the first vaccination the patient had had performed in his life.

During the period between 17th to 25th March, the patient appeared perfectly fit, and was up and running about. He was apyrexial during the whole period. On the afternoon of 25th March he felt unfit, complaining of a headache, and he was found to have a temperature of 101 F and was said to have vomited on several occasions.

The following morning he was found to be semi-comatose and on examination his temperature was 97° F., his pupils were equal and contracted to light, he had no neck rigidity and Kernig's sign was negative. There was marked hypotonia of the limbs, but there was no paralysis The arm jerks, biceps and radial, in addition to the right ankle and right knee jerks were diminished, the left ankle jerk was exaggerated and there was left ankle clonus, but no left patellar clonus. The left plantar gave an extensor response, but no response was elicited on the right side Three large vaccination crusts were noted on the left upper arm, these were drying up, but had been scratched and had become to some extent secondarily infected. A lumbar puncture was performed. The fluid was found to be clear under no increase of pressure and contained 13 cells per c.mm. these were all lymphocytes. His temperature rose to 103° F that evening and he died at 6 p.m.

A postmortem of the skull and brain only was performed the following day No macroscopic change was observed in the dura, and no pus was present. The blood vessels on the surface of the brain were markedly injected, the hyperaemic vessels giving an appearance of small spider-like ramifications extending all over the surface of the brain and down into the sulci. The cross-section of the cerebrum, cerebellum and medulla

revealed no noticeable pathological changes. The report on sections of the brain was "an encephalitis is present which corresponds in appearance to that described in post vaccinial cases."

Discussion

The following is a summary of the findings in regard to post-vaccinial encephalitis extracted from the report of the Committee on Vaccination, 1928, and also embodying the findings in the report of the ANDREWES Committee 1923

Incidence—The rough incidence of the disease in England between November 1922, and October 1927 was one case in 48 823 vaccinations A remarkable contrast to this was found in Holland during the periods 1924 and 1926 where the incidence was one case in 4 028 vaccinations. This enormous difference might, in part, be due to the fact that it was customary in Holland to defer the primary vaccination until the 3rd 4th or 5th years of life

Primary Vaccination—Of the twenty-five cases reviewed by the Committee on Vaccination, twenty one cases had not been vaccinated previously three cases had previously been vaccinated on one occasion, while in one case a previous vaccination was alleged, but no evidence of it could be found. Of the first eleven cases (Nos 1 to 11) reviewed in the report of the ANDREWES Committee, all, with one exception, were primary vaccinations Of the total therefore, thirty-one cases, or 86 1 per cent. were primary vaccinations four cases or 11 1 per cent. were secondary vaccinations, and in one case (2 7 per cent.) no marks could be found to substantiate the history of previous vaccination.

Age incidence—The average age incidence was 11 1 years the highest age recorded being 50 and the youngest 1 month 74 5 per cent. of the eighty six cases occurred between the years of 6 and 15

Incubation period—The incubation period was found in ninety four out of 125 cases collected from British and Continental sources to lie between the 9th and 13th day after vaccination the most favoured day being the 11th.

Sex incidence—Females were affected in the majority in the ratio of five females to three males.

Fatality rate—Out of eighty seven cases reported from both Committees 48 deaths occurred. This is a mortality of about 55 per cent.

Symptoms and signs—The chief signs and symptoms described in their order of frequency were stupor (88 per cent.) headache (54 per cent.) vomiting (46 per cent.) and pyrexia (43 per cent.) Next in frequency were incontinence of urine (30 5 per cent.) head retraction (21 per cent.) convulsions (18 per cent.) spastic paralysis (17 per cent.) positive Babinski (15 per cent.) and positive Kernig (14 per cent.) Among the other less frequent findings were flaccid paralysis (9 7 per cent.) strabismus (9 7 per cent.) retention of urine (8 per cent.) ankle clonus (7 per cent.) and paresis (7 per cent.) In 17 per

was also involved. The nails were hypertrophied and deformed. There was infiltration of the skin, both of the nail folds and beneath the free ends of the nails, giving a club-shaped appearance to the fingers and thumb

Feet   There was a deep erosion of the plantar surface of the right heel. There were fissures beneath the heads of the third fourth and fifth metatarsal bones, and over the plantar surfaces of the toes. There was slight cutaneous thickening of the outer parts of the soles, but the medial longitudinal arches were almost unaffected. There was a state of onychia and paronychia of the toes, resembling the corresponding condition of the fingers.

The Kahn Test was + + +

A general examination revealed no other sign of yaws. Nothing abnormal was found in heart, lungs or nervous system.

Progress

Recovery commenced within 4 days of the first injections, which were given on 12th March. After 8 days the pain had almost disappeared. Within 20 days all subjective symptoms had vanished and there was an obvious improvement in the appearance of the parts the skin was less thickened, the fissures were healing and the unhealthy cuticle was peeling off. The second series of photographs were taken on 27th April, after the administration of seven weekly injections of 0·45 gramme N.A.B intravenously and 0 2 gramme beneath intramuscularly They reveal a marked improvement. Fig 6 may at first sight give the impression that a fresh ulcer has broken out on the lateral aspect of the right heel. This depression is, however the result, not of new disease, but of exfoliation of unhealthy epithelium which was present before the commencement of treatment, as can be seen by a study of Fig 5

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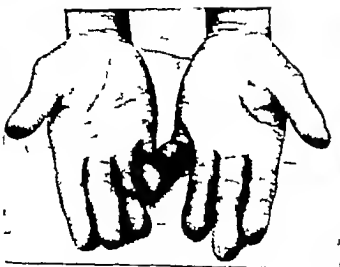
BEFORE TREATMENT   AFTER TREATMENT

A CASE OF FOOT YAWS

ANAVENOMS AND THEIR USE IN THE PREPARATION OF ANTIVENOMOUS SERA

Polyvalent Anti-*Bitis arietans*-*Naia flava* Serum and Specific Antivenenes against African Viperine and Colubrine Venoms

BY

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In a previous publication (GRASSET and ZOUTENDYK, 1933) it was shown that it is possible by the use of formolized venoms detoxicated under optimum conditions to set up a method by which large doses of atoxic venom derivatives or anavenoms* can be administered safely to horses to obtain in a period of 2 to 3 months antivenomous sera of high potency

Introduced at the South African Institute for Medical Research in 1932, the method has been used during the past 10 years for the production of colubrine, viperine and polyvalent therapeutic antivenenes.

We intend in the present paper to give an account of the results obtained. Special reference will be made to the preparation of anavenoms their use in horse immunization, anti viper and cobra titres in a series of horses used for the preparation of polyvalent South African antivenene. We shall also refer to various immunological investigations undertaken with the aim of perfecting the method, and its application to the preparation of other types of antivenenes

PREPARATION OF ANAVENOMS

For practical reasons our investigations dealt originally with African viperine and colubrine venoms Subsequently they were extended to the detoxication of a variety of Asiatic venoms These further studies proved at a later date to be of great benefit, as will be seen in the substitution of African *Naia flava* venom by Indian *Naia tripudians* venom (and anavenom) in the preparation of polyvalent antivenene against African colubrines

Concentration of venom solution used in detoxication

While a considerable amount of immunological work has been carried out with 2, 4 or 10 per cent. solutions of venoms the 1 per cent. solution was used as the usual concentration both for viperine and colubrine venoms

* Term proposed for these antigens by RAMON (1925)

This relatively strong concentration of 10 mg. per c.c. was chosen as the most convenient in the detoxication process, as well as for hyperimmunization purposes. The maximum volume of polyvalent anavenom injected at the end of the scheme of immunization does not, under such conditions, exceed a total volume of 400 c.c.

Higher concentration of venoms such as 2 and 4 per cent. can be used shortly after their detoxication but offers technical disadvantages, certain physio-chemical instability and the formation of gels. 10 per cent. concentration requires too high a proportion of formol, resulting in a rapid coagulation of the venom proteins.

Proportion of formol.

For each venom the optimum amount of formol (40 per cent.) was determined experimentally so as to obtain detoxication of the toxic properties, while keeping as intact as possible their antigenic properties. This optimum proportion of formol varied widely from 0 4 to 1 per cent. according to the nature of the venom—viperine or colubrine, and other conditions under which detoxication was carried out. These conditions included —

1 Concentration of venom.
2 Biochemical constitution of solvent medium used for detoxication.
3 pH and buffer
4 Temperature.
5 Period of detoxication.

VIPERINE ANAVENOMS

As shown in our original paper detoxication of a variety of African viperine venoms was studied and anavenoms for the following viperines were obtained —*Bitis arietans Bitis cornutus Bitis caudalis Causus rhombeatus* similar results subsequently being obtained for *Bitis gabonica* and *Bitis nasicornis*.

Bitis arietans ANAVENOM

For practical purposes, we shall limit ourselves in the present paper to the technical description of the preparation of *B arietans* anavenom. Immunological investigations have shown that from the antigenic point of view this venom is to be considered as the prototype of Southern African viperines. It possesses, indeed, the highest and the widest antigenic group action (GRASSET ZOUTENDYK and SCHAAFSMA, 1935) This is evidenced by the high specific and group neutralization exerted by monovalent anti *B arietans* serum against venoms of the more common of Southern African viperines such as *B caudalis* and *Causus rhombeatus*.

The wide distribution of *B arietans* commonly known as puff adder in the Southern Africa hemisphere and the relatively large proportion of bites for which this species is responsible constitute further reasons for its selection

as group viperine antigen in the preparation of polyvalent South African anti-venene

Experimental work has shown that 1 per cent. solutions of *B arietans* in saline is rendered atoxic by treatment with 0·6 per cent. formol after a period of 30 days at 38° C   Guineapigs can tolerate the subcutaneous injection of 3 c.c of the detoxicated product corresponding to 30 mg of the original venom, and rabbits 50 mg intravenously i e 50 m.l d. of the original venom.

After receiving four injections of *B arietans* anavenom, a total of 250 mg rabbits so immunized can stand the intravenous test dose of 15 to 20 mg of *B arietans* venom, i e 15 to 20 m.l d. for the controls—1 c.c. of serum of the immunized rabbits neutralizes in vitro from 1 to 2 mg of the same venom.

For hyperimmunization of horses for antivenene production we use *B arietans* toxoid treated with a somewhat lower percentage of formol such as 0 4 per cent. Whilst the injection of this antigen is followed by a slight local swelling it can be safely injected in rapidly increasing doses and gives on the whole a better immunity response than the anavenom treated with 0·6 per cent. formol.

For preparation of a batch of anavenom a pooled mixture of 60 grammes of *B arietans* venom is made from specimens of venom of this viperine obtained from various geographical sources in South Africa Rhodesia, Tanganyika, Kenya and the Congo This tends to minimize the risk of excluding possible variety in the antigenic constitution and differences due to other factors such as seasonal moulting periods or repeated milking in captivity   Such factors are known to have an appreciable influence on the toxicity and antigenicity of venoms even of the same zoological species A 1 per cent. solution of venom is prepared in 6 litres of saline and heated to a temperature of 30 to 40° in order to accelerate the dissolution. The venom solution is added to 0·4 per cent. of formol (40 per cent.) and after thorough shaking the slightly opalescent solution is incubated for 30 days at 37° C

At the end of this period, the product is clear slightly opalescent with a pale whitish deposit of organic debris at the bottom of the flask. It is then removed to a refrigerator until required for use

Bacteriological controls of detoxicated venoms show that this amount of formol is sufficient to sterilize anavenoms from aerobic and anaerobic organisms commonly found in dry venoms

Researches on the stability of viperine and colubrine anavenoms have shown that anavenoms keep their antigenic powers for a period of over a year if stored at a temperature of from 2° to 40 C (GRASSET and ZOUTENDYK, 1935) For long periods of storage it is advisable to dialyze anavenoms in order to delete free formol.

COLUBRINE ANAVENOMS.

In contrast to the venoms of most viperines such as *B arietans* and *B cornutus* which are readily detoxicated in saline, venoms of African colubrines

such as *Naia flava* and *Sepedon haemachetes* remain toxic even after several months of incubation at 37° in contact with 1 per cent. formol. GRASSET and ZOUTENDYK (1933) have shown that detoxication can be achieved by 0·8 per cent. formol in 30 days by substituting saline with hydrolysed peptic medium as a solvent and medium of detoxication of the venom. In practice we found that Martin's broth (hydrolysed pig's stomach extract and beef infusion) is quite suitable for this purpose.

By applying the same process to venoms of other African colubrines, the venom of *Naia haie Naia nigricollis Sepedon haemachetes* and *Dendraspis angusticeps* have been similarly detoxicated with 0·7 to 0 8 per cent. formol in Martin's broth in the time period of a month at 37° and converted into specific antivenoms

Investigations on Asiatic colubrine venoms have shown that detoxication of *Naia tripudians* is also considerably accelerated by the same technique. After a period of 30 days of detoxication by 0 8 per cent. formol in Martin's broth, 30 mg of the resulting *N tripudians* antivenom can be safely injected in guineapigs. By contrast the same amount of venom, treated with the same percentage of formol, during the same period, will kill the guineapig in 1 hour Full detoxication is only reached in 2 months, *i.e.* double the length of time as compared with antivenom prepared in broth.

PREPARATION OF *Naia flava* ANTIVENOM FOR HORSE IMMUNIZATION.

For immunological reasons similar to those referred to above in connection with the venom of *B anietans* the venom of *Naia flava* has been selected from South African colubrine venoms as a prototype antigen for the preparation of polyvalent antivenene.

A 1 per cent. solution of *Naia flava* venom is prepared by dissolving 60 grammes of this venom in 6 litres of Martin's broth heated to a temperature of 30 to 40° C This solution is added to 0 8 per cent. formaldehyde and incubated at 37° The addition of formol is followed, in the absence of pH adjustment, within a few minutes by an opalescence which increases during the subsequent hours. After 24 hours, throughout the product a fine precipitate is observed which settles gradually and leaves a clear layer at the upper part of the antigen. Within 3 to 4 days the precipitate has condensed into a coarse, straw like deposit occupying the lower portion of the flask of antigen the upper portion is practically clear and a pale yellow colour After 30 days incubation the antigen is well shaken in order to break down the heavy precipitate to a fine suspension. Four to 5 c.c. of this product, *i.e.* 40 to 50 mg. representing 200 m.l.d. doses of the original venom, injected subcutaneously into guineapigs proves atoxic, producing only local oedema. Fifty c.c. *i.e.* 500 mg of antivenom can be injected similarly into normal horses without ill effects other than local swelling

Rabbits submitted to four injections of 50 50 75 and 100 mg of *N flava*

anavenom are then able to resist the intravenous injection of 4 mg of venom of *N flava* representing 20 m.l.d. for the controls

Immunization of guineapigs and rabbits respectively with the soluble and precipitated fractions of *N flava* anavenom shows that these two fractions are both antigenic.

In practice the precipitated nature of the anavenom does not interfere with the immunization of horses, even when large doses are injected such as 1 600 mg at the end of immunization In fact, it has a beneficial effect on the immunity response, as other antigens have when injected in a precipitated form, e g diphtheria or tetanus alum precipitated toxoids, delaying the absorption of the antigen and stimulating local inflammatory actions This results in a better utilization of the antigen by the system and ultimately in a higher immunity response This action is further increased by the addition of tapioca (or sago) to the antigen. It is added in the form of a fine sterile powder a few hours before injection of the anavenom according to the method of RAMON (1925a)

We have used this accessory method of immunity stimulation during the last 15 years for tetanus and diphtheria production and have found it also beneficial in the case of antivenene production. This is particularly the case in anavenoms obtained in a non precipitated form, as is usual in those derived from viperine venoms and also from the Indian *Naia tripudians* which remains in a soluble form even in broth. This stimulating action is particularly evident in horses immunized originally with plain anavenoms, the titres of which were gradually dropping After re immunization with the same antigens to which tapioca had been added they showed a new rise in neutralizing titre for a considerable period. The use of tapioca as an accessory stimulant in antivenomous immunity response has also been found beneficial by MALLICK (1935) in the preparation of anti *Naia tripudians V russelli* serum using non-modified venoms as antigens

Role of Buffer and pH in the Preparation of Anavenoms

Naia flava venom. The role of buffer in the detoxication of the venom of this colubrine is mainly reflected in the physical conditions of the resulting anavenom.

As shown above, the formolization of *Naia flava* venom results in a heavy precipitate in the absence of a buffer particularly if done in broth. Martin s broth used for the solution of venom was added to Glenny s buffer used in the Schick test, i e borsacic acid 5 25 grammes sodium bi borate 3 56 grammes, and sodium chloride 6 1 grammes per 1 000 c c. of broth. This results in a delay and reduction in the amount of precipitate after addition of formol compared with the product with no adjustment It was thus tried to increase the amount of buffer The addition of the double normal concentration of the same buffer was found to inhibit completely the precipitate of the resulting

anavenom, which kept, even after several months storage its light yellow colloidal appearance.

The same results were obtained with *Sepedon haemaketes* anavenom, i.e. a heavy precipitate without buffer a reduced precipitate on the addition of a normal concentration buffer strong opalescence and colloidal appearance with double the normal concentration of buffer

As will be seen later this soluble form of N *flava* anavenom was used for the intravenous immunization of horses in doses up to 200 c.c. (2,000 mg. of the original venom)

Role of pH in *Naia flava* venom detoxication.

The Beckman pH electrometer was used in all pH determinations referred to in these experiments.

The following experiments were carried out on a batch of 2,000 c.c. of the product under test. The pH of the original Martin's broth used for the venom solution was 7·6 The solution of 20 grammes of *Naia flava* venom does not much alter the pH After the addition of a concentration of 0·8 per cent of formaldehyde (40 per cent.) the pH dropped to 7·1 (the pH is liable to drop in such conditions to below 7 as ascertained in other batches of the product) The medium was thereafter distributed in a series of flasks each containing 100 c.c. of the product and the pH was adjusted respectively to 3 4 5 6, 7 8 and 9

The series of flasks was then incubated at 37 In all flasks during the following hours an opalescence was observed, which reached a maximum at both extreme acidity and extreme alkalinity This was followed in the flasks of pH 3 and 4 by an early precipitate which rapidly settled down to the bottom of the flasks, leaving a clear fluid above A much finer precipitate was observed in the flasks of pH 5 and 6 which gradually settled to the bottom. Flask pH 7 showed a very strong opalescence and remained so with only a slight precipitation (This may be followed in the absence of a buffer by the formation of a heavy precipitate of variable amount from batch to batch.) In flasks of pH 8 and 9 a heavier and more rapidly forming precipitate was observed leaving a clear medium as occurs in the case of high acidity

The abundance of the precipitate throughout the series of N flasks varied also according to the origin of the N *flava* venom. The determination of the rate of detoxication according to the pH has been done on several specimens of N *flava* venom—samples of the venoms being taken after 10, 20 30 and 40 days of detoxication. A sample adjusted to pH 9 was found to be already atoxic after 20 days. Of this 2 c.c. could be subcutaneously injected into guineapigs without ill effects. The same dose of formolized venom of pH 8 was fatal in 2 hours. Atoxicity for the sample pH 8 was reached after 30 days and after 50 days for that of pH 7 2 c.c. of a sample of pH 6 was found to be still toxic after 60 days incubation, killing guineapigs in 2 hours.

Active immunization with the products corresponding to pH 6, 7 8 and 9

showed that more speedily detoxicated, formolized products of pH 8 and 9 had lost part of their antigenic powers. In practice large batches of *N flava* venoms to be converted into anavenoms are adjusted to pH 7 to 7·4

The same pH influence has been found to hold in the case of the detoxication of the venom of *N tripudians* as well as in viperine venoms such as *B arietans* alkalinity accelerates detoxication while acidity delays it. The optimum is round about neutral point.

CONTROL METHOD FOR ATOXICITY OF ANAVENOMS BASED ON DISAPPEARANCE OF ORIGINAL HYPERGLYCAEMIC PROPERTIES OF VENOMS

Besides general atoxicity as ascertained by intravenous subcutaneous and intradermal injections in experimental animals, an additional test for checking atoxicity is afforded by the disappearance of hyperglycaemic properties produced originally by the respective venoms before their detoxication

BERTRAND and VLADESCO (1940) have given evidence of the hyperglycaemic action exerted in the blood of experimental animals injected with a variety of colubrine and viperine venoms Researches done in this department, details of which will be postulated in another publication, on *Naja flava* and *Bitis arietans* venoms confirm these findings

Comparative experiments with these venoms and their respective anavenoms showed that after full detoxication the resulting products failed to produce such hyperglycaemic action any longer even if injected in considerable amounts

Naja flava VENOM AND ANAVENOM

The intravenous injection of rabbits of 2,000 grammes weight with 0·35 mg of *N flava* venom (1 m.l.d. in 4 to 6 hours) was followed by a considerable increase in blood sugar—from 70 to 80 mg before injection up to 140 to 160 mg per 100 c.c. 2 to 3 hours later before the death of the animals In some animals (rabbits) injected subcutaneously with 1 mg of the same venom, a four to five fold increase was observed, e g from 60 to 290 mg

After a month of detoxication with 0·8 per cent. formol (in broth plus buffer) the intravenous or subcutaneous injection of 5 c.c. of the resulting anavenom corresponding to 50 mg of the original venom or 150 m.l.d. was followed only by inappreciable changes in the blood sugar of these animals from 50 to 65 mg before injection to a maximum of 65 to 70 mg per 100 c.c. after 4 to 6 hours, and down again to 65 to 70 mg per 100 c.c. after 24 hours, with no ill effects.

Bitis arietans VENOM AND ANAVENOM.

Similar effects have been found with *B arietans* venom and its corresponding anavenom as illustrated in the following experiment

A rabbit of 2 000 grammes was injected intravenously with 1·4 mg of *B arietans* venom. Death followed after 4 to 6 hours The blood sugar 5 minutes before injection, was 50 to 60 mg per 100 c c. Three to 4 hours

after injection it increased to 250 to 310 mg *i.e.* there was a five to six times increase in blood sugar

Rabbits of 2,000 grammes were injected intravenously with 3 c.c. of *B. arietans* anavenom (detoxicated for 1 month with 0·6 per cent. formol). The blood sugar 5 minutes before injection was 50 to 60 mg per 100 c.c. Four to 6 hours afterwards it reached a maximum of a 100 and after 24 hours came down again to 60. The rabbits survived without ill effects.

Partially or imperfectly detoxicated venoms injected according to the same technique resulted in a late death of the animals. Such products will produce hyperglycaemia within appreciable limits but less than in the case with the original venoms. Three c.c. of *B. arietans* venom detoxicated with 0·6 per cent. formol and injected after 30 days incubation into rabbits of 2,000 grammes, gave the following results. The blood sugar 5 minutes before injection was 80 mg per c.c. Six hours later it rose to 125 mg. The animal was sick and death followed sooo after.

This method, therefore, constitutes an additional and sensitive test, comparable to the general atoxicity test and suitable also for checking the atoxicity of anavenoms or the residual toxicity of incompletely detoxicated venom derivatives.

PREPARATION OF POLYVALENT *Bitis arietans-Naia flava* ANTIVENENT

For the immunological reasons expressed above the venoms of *B. arietans* and *N. flava* have been selected as the respective prototypes of viperine and colubrine venoms in the production of polyvalent antivenene for Southern Africa.

The immunization of the horses entails weekly subcutaneous injections of the combined *B. arietans-N. flava* anavenoms according to the following schedule —

TABLE 1

Injection.		*B. arietans* ana. venom.		*N. flava* anavenom.	
No.		Mg	Vol. in c.c.	Mg	Vol. in c.c.
1	.5	*i.*	5	.5	*i.*	2·5 + tapioca
2	100	*i.*	10·0	100	*i.*	10·0 + tapioca
3	200	*i.*	20·0	200	*i.*	20·0 + tapioca
4	400	*i.*	40·0	400	*i.*	40·0 + tapioca
5	600	*i.*	60·0	600	*i.*	60·0 + tapioca
6	800	*i.*	80·0	800	*i.*	80·0 + tapioca
7	1,200	*i.*	120·0	1,200	*i.*	120·0 + tapioca
8	1,600	*i.*	160·0	1,600	*i.*	160·0 + tapioca

The two anavenoms are mixed in equal parts according to the dosage required. The mixture has tapioca added on the evening before the injection

and is left at room temperature overnight, or alternatively the tapioca is added a few hours before injection to the anavenom which is then warmed to 35° to 40° C, and shaken well before injection

Reactions following injections of anavenoms are of a mild protein nature —slight local oedema after the first inoculation without haemorrhagic lesions or neurotoxic symptoms The severity of the swelling increases with the dosage and is partly due to the tapioca Occasionally the formation of sterile abscesses is observed, which are punctured when formed.

General reactions are limited to thermal reactions These are negligible at the commencement of immunization, but may reach 103° to 104°F at the end of immunization They usually show a sharp rise, reaching a maximum on the evening of the injection and settling down within 48 hours without those neurotoxic or nephritic complications which too often are observed when non-modified venoms are used as antigens.

We came across horses which appeared unduly sensitive to detoxicated venom proteins themselves, and showed protein shock, as a rule towards the end of immunization In such cases it is advisable to split the doses into two or three fractional injections spread over 1 or 2 days, accompanying them with a heart stimulant, e g, camphorated oil.

Titration.

A sample of blood is taken from the horses undergoing immunization on the 7th day after the last injection of anavenoms for the determination of anti B *arietans* and *Naia flava* neutralizing titres If 3 c.c. of such serums are found to neutralize *in vitro* a minimum of 10 mg B *arietans* and 1 mg of N *flava* venoms the horses are bled on the following day as follows —

| 8th day | 1st bleeding | 10 litres |
| 10th day | 2nd bleeding | 10 litres |

During the first years of these studies determination of the anti B *arietans*-N *flava* neutralizing titres were carried out on rabbits, until the titration method at different levels on mice was introduced. With a view to maintaining homogeneity in the exposure and interpretation of these serum titration results over the last 10 years all serum titration results given in the present paper will be expressed by means of the same rabbit titration method. After the correction introduced in the interpretation of these essays titration results on rabbits compare closely with those obtained when using mice, according to the standard method proposed by the Standardization Committee of the League of Nations (GRASSET 1940)

Technique

Three c.c. of immunized horse serum under test are mixed with varying doses of B *arietans* venom from 10 to 20 mg and of N *flava* venom from 1 to 2 mg The great majority of viperine and colubrine neutralizing titres fall within these limits. After an hour of contact at 37° these mixtures (after the addition

of saline to bring the volume of each up to 10 c.c.) are injected intravenously into rabbits of 2,000 grammes. If the minimum titre of 10 mg of *B arietans* and 1 mg of *N flava* venom is not reached the horses receive a supplementary injection of 1 600 mg of antivenom, *i.e.* 160 c.c. of *B arietans* and *N flava* antivenom, which usually brings the serum to the required titre.

The *B arietans* and *N flava* venoms used for these titration essays consist of pulverized pooled venoms, which are kept specially for titration purposes for a period of several years in glass stoppered containers in the dark. Their toxicity is compared periodically

Let us mention in passing an interesting point regarding the flocculation phenomenon in *B arietans* venom-antivenom mixtures. When series of mixtures are prepared to correspond closely to the neutralisation range an opalescence is observed which appears usually within a few minutes after their preparation. This opalescence increases rapidly and usually results in an "original" flocculation as in the case of diphtheria toxin-antitoxin mixtures. Care must be taken to break up this precipitate by drawing the mixture in and out of the syringe several times before giving the antivenous injection. If such precautions are not taken, the heavy flocculation formed in the mixture frequently results in a violent shock to the rabbit immediately after injection, heavy dyspnoea and collapse very often ending in death.

A similar flocculation is observed quite frequently in the *N flava* venom serum mixtures but is usually delayed, taking place after a few hours and is of a less heavy character In unpublished investigations by the author on the possible use of this phenomenon in a titration method, the same conclusion was reached as that expressed by CALMETTE (1908) in the case of *N tripudians* venom-antivenene titration. The phenomenon of original flocculation in specific venom-antivenene mixtures takes place in a zone close to the neutralisation point but does not allow such a close accurate titration as that obtained from the inoculation, with the same series of mixtures, of experimental animals such as mice.

The immunity response to *B arietans* as in the case of anti viperine sera compared with that of colubrine is considerably quicker and higher than the response to *N flava* The minimum neutralising titre of 10 mg *B arietans* is often considerably exceeded, *e.g.*, 14 mg after the eighth injection of 1,600 mg, while an extra injection of 2,000 mg is in some cases required to bring colubrine antibodies to the minimum neutralising titre.

Re-immunisation.

After bleeding the horses are given a period of from 4 to 6 weeks rest before proceeding with re-immunisation. This consists of three subcutaneous injections of mixed *B arietans-N flava* antivenoms given at weekly intervals as follows —

TABLE II.

Injection	*B arietans* antivenom.		*N flava* antivenom.	
No.	Mg	Vol. in c.c.	Mg	Vol. in c.c.
1	400 *i.e.*	40	400 *i.e.*	40 + tapioca
2	800 *i.e.*	80	800 *i.e.*	80 + tapioca
3	1 600 *i.e.*	160	1 600 *i.e.*	160 + tapioca

Blood samples for titration are taken 7 days after the third injection This is followed by a bleeding of 10 l, the following day if neutralization for *B anetans* and for *N flava* is found satisfactory A second bleeding of 10 l is carried out 3 days later In the great majority of the horses these three injections are sufficient to bring the serum to its original titre, more particularly in the case of *B anetans* Subsequent courses of 4 to 6 weeks' resting periods re immunization and bleedings are planned according to the above schedule

Thus an average of four to five re immunizations and corresponding bleedings are obtained per year from each horse, representing an annual volume of 40 to 50 l of antivenene. This is obtained without undue strain on the horse's condition and as can be seen in Table III p 476 many horses have been kept on antivenene production for periods of from 2 to 3 years.

While for the first immunization it is advisable to complete the whole course or at least the first six injections using anavenoms—for re immunizations, partly detoxified venoms (2 to 3 weeks at 37°) may be used in cases of necessity Notwithstanding increased local reactions, abscess formations and a sharper rise in temperature, this procedure does not endanger the life of the horse. Heart stimulants are given as preventive measures in such instances Such procedure, however necessitates in many cases a longer period of rest between re immunizations

Certain horses reach their maximum anti-colubrine and anti viperine titres at the end of the first immunization, while others attain this only after one to two re-immunizations. The latter is more frequently the case and holds to a greater degree for anti-colubrine immunizations These neutralizing titres may then remain within small variations at the same high level for long periods —in some horses for several years. The cobra immunity is usually the first to weaken.

When signs of a decline in the anti toxic response to the usual antigenic dosage are observed, the re immunization of the horse is increased by an extra dose or supplementary injection of 2,000 mg of the anavenom corresponding to the type of antibody-colubrine or viperine in decline We have found no practical utility in increasing higher than 2,000 mg either the colubrine or viperine anavenoms. This extra dosage usually results in a reinforcement of the immunity and an appreciable rise in the neutralizing titre for one or several re immunizations. Care must be taken in such conditions to lengthen the periods of rest between immunization to allow the healing of the larger abscesses which often follow the administration of these increased doses When in spite of these supplementary doses the titre drops to 8 mg *B anetans* or 0 8 mg *N flava* the limit below which serum is rendered unsuitable for therapeutic use even after concentration the animal is definitely discarded.

During the period 1932 to 1943 a total of forty-six horses was used for the production of polyvalent antivenene In all of these, immunization and subsequent re immunizations were carried out with *B anetans* and *N flava* (or *N tripudians*) anavenoms according to the above described method.

Data regarding the respective neutralizing titre against *B antetans* and *N flava* venoms for each of these horses are included in Table III. Columns 2 and 3 show for each horse the respective anti *B antetans* and *N flava* neutralizing titres (maximum titres obtained during immunization or re immunization) expressed in mg of the respective venoms per 3 c.c. of serum. Results tabulated in columns 4 and 5 express the actual neutralizing titre determined in mg per c.c. of serum of the *B antetans* and *N flava* venom, after " correction " and after taking into account the amount of venom tolerated by the animal used for the test, in the absence of antivenene.

As shown by BANIC and LJUBETIC (1938) in their study on the standardization of European viper serum about four fifths of the amount corresponding to a c.l.d. of venom for the animal under test can be tolerated by its system without the necessity for neutralization by the specific anti-serum. The additional one fifth lethal dose is the amount of venom which will result in the death of the animal. To determine therefore, the correct amount of venom neutralized in antivenene essays, one must first subtract from the direct figure the amount corresponding to four fifths of the c.l.d. for the experimental animal under test. In the present case this corrected figure is obtained by subtracting from the amount of *B antetans* and *N flava* venom neutralized by 3 c.c. of serum, the amount corresponding to four fifths of one certainly lethal dose (c.l.d.) of the respective venoms, for a rabbit of 2,000 grammes. By dividing this corrected figure by three the actual titre per c.c. of serum is obtained.*

EXAMPLE.

Serum of Horse 125

A **Determination of B arietans neutralizing titre**

1 c.l.d of *B arietans* venom for intravenous injection to rabbits of 2,000 grammes = 1 3 mg

4/5 of c.l.d = 1 mg

Amount of *B arietans* venom neutralized by 3 c.c. of serum = 15 mg

15 mg *B arietans* − 1 mg (4/5 c.l.d.) = 14 mg

14 mg ÷ 3 c.c. serum = 4·66 mg = titre of neutralization per c.c. of serum.

The introduction of this correction in the technique of antivenene titration allows a direct titration of antivenene in terms of the weight of venoms and can be used with any sample of venom, as long as the c.l.d. of that venom is determined on the animal species of the same weight. This factor has been taken into account in the standardization method of anti-European viper serum (modification of Banic Ljubetic titration method on mice at different levels) proposed by IPSEN (1938) to the Permanent Standardization Committee of the League of Nations. It has been shown that this method can be applied with satisfactory accuracy in the standardization of both *Bitis arietans* and *Naja flava* antibodies contained in the respective monovalent sera or in the resulting polyvalent antivenene (GRASSET 1940).

B Determination of N flava *neutralizing titre*

1 c.l.d. of *N flava* venom for intravenous injection of rabbits of 2,000 grammes = 0 35 mg

4/5 of c.l d. = 0 28 mg

Amount of *N flava* venom neutralized by 3 c.c of serum = 1 5 mg

1 5 mg — 0 28 mg (4/5 c.l d.) = 1 22 mg

1 2 mg + 3 c.c. serum = 0 406 mg = titre of neutralization per c.c. of serum.

As can be seen, the difference ratio brought about by this correction is relatively not so important in the case of *B arietans* serum titration for which the neutralization per c.c. of natural serum is high—from 3 to 6 mg of venom per c.c. In the case of Horse 125 referred to in Table III it is in the proportion of 1 15 = 6·6 per cent. By contrast, this correction is more noticeable in the results of the *N flava* serum for which the ratio of specific neutralization expressed in mg of venom is usually ten times lower than that for *B arietans* For the same polyvalent Horse 125 the difference in the *N flava* titre is 0 28 1 5 = 18·6 per cent The ratio of the correction quotient is therefore about three times higher for *N flava* than for the *B arietans* titre In practice a serum such as that of Horse 125 which without correction apparently neutralizes 0 5 mg of *N flava* venom per c.c. is found after correction to neutralize actually only 0·406 mg

The technical reason why in these rabbit titrations, especially in *N flava* essays we have kept to 3 c c. serum, is to work with reasonably high test doses of venom i e 1 to 2 mg of *N flava* venom, representing about 3 to 6 c.l.d. for the controls The use in the tests of smaller volumes of natural anti *N flava* serum would tend to reduce the corresponding test doses of venoms to limits too narrow to permit of accurate interpretation of the essays

For concentrated antivenene which usually possesses three times the potency of the original serum, the differences brought about by the correction are relatively not so high Thus for a concentrated globulin which neutralizes 14 mg of *B arietans* and 1 3 mg *N flava* per c.c. the titre after concentration will be 13 mg and 1·02 mg for the respective venoms

Analysis of the corrected titres given in columns 4 and 5 of Table III for the sera of 46 horses shows that the *B arietans* titre varies from 3 to 6 3 mg per c.c. of serum with an average of 3 73 mg (4 mg uncorrected titre) per c.c. For *N flava* the titre varies from the exceptionally low figure of 0 15 to 0·64 mg with an average of 0 32 mg (0 4 mg uncorrected titre) per c.c. These titres represent conservative figures as in many cases neutralization could not be completed to end titre, but was taken on a minimum requirement basis due to shortage of animals.

These figures reflect also the different types of immunity response which characterize anti-viperine and colubrine immunity They are the consequence of the different antigenic natures of the antigens. The complex enzyme like

TABLE III

SHOWING ANTI-B SYSTEM AND λ flava NEUTRALIZING TITRES OF 45 HORSES BEFORE AND AFTER CORRECTION — ALSO THE NUMBER OF COURSES AND THE PERIODS OF IMMUNIZATION IN ANTIVENENE PRODUCTION.

No of horse	Neutralizing titre per 3 c.c. of serum in mg of enom.		Neutralizing titre per c.c. of serum after correction.		Number of courses of immunization.	Period horse in use for antivenene production.
	B. structus.	λ flava.	B. structus.	λ flava.		
						Yrs. Mths.
105	1	1 3	3 66	0 106	4	1
117	1	1 3	2 66	0 406	6	1 6
123	14	6	3 64	0 673		8
1 5	1	1 3	4 66	0 406	10	4
156	40	monor B anet ser	6 23			
147	1	1 3	2 66	0 406	3	
158	15	1 3	4 06	0 406	2	1 3
163	10	1	3 0>	0 306	4	2
170	1	1 0	3 66	0 21	10	3
179	1 1	1 0	3 0>	0 4	3	1
18	11	1 3	4 66	0 406	11	3
18	1	1 3	2 64	0 406	3	1 16
227	monor λ flava ser	1 0		0 24		
201	12	1 3	3 66	0 406	1	4
209	10	0 8	3 0>	0 172	6	1 6
279	10	0 8	3 0	0 172		6
279	1	1 0	3 0>	0 4	4	1 6
241	15	1 5	4 66	0 406	13	3
243	10>	0 9	3 0>	0 406		6
313	10	0 8	3 0>	0 172	3	1
343	10>	1 4	3 0>	0 373	9	2
311	10	1 9	3 0	0 306	17	3 3
312	1	1	3 66	0 304	47	6
320	10	3 0>	0 35	13	6
3	1	1 6	3 66	0 44	14	4
233	10	1 0	3 0>	0 306	3	1
230	1	1 1	3 0	0 273	14	
231	10>	1 0	3 0>	0 4	7	1 6
31	1	3 0	3 66	0 4	3	1
304	13	3 0	4 0	0 4	16	3
365	13	1 4	4 66	0 373	1	3
271	1 1	0 7	3 0	0 143	15	1 7
272	16	1 3	3 0	0 24	16	3 4
333	10>	0 9	3 0>	0 406	1	4
330	10 >	0 9	3 0>	0 406	1	5
292	17	2	3 3	0 81	1	2 10
293	16	0 8>	6 0	0 206	1	7
295	10	0 8	3 0	0 173		3
396	10	0 8	3 0	0 173	3	1
369	10	0 8	4 0	0 406		3
403	12	1 0	4 66	0 4	6	
435	19	2 9	6 0	0 64	4	2
436	12	1 3	3 66	0 24	8	1
441	11	1 3	3 23	0 373	8	6
449	40	1 4	3 33	0 373	7	1
454	16	1 0	4 66	0 24	3	1

properties which mostly characterize viperine venoms give a considerably higher antibody response as compared with the neurotoxic response in colubrine venom. The neutralization ratio for these two types of antibodies expressed in mg of the respective venoms per c c. of serum is about 10 to 1

It is also interesting to note that on the whole the individual immunity response for the respective horses is relatively of the same magnitude for the two types of viperine and colubrine antigens. Thus if we consider the neutralizing figures for Horses 123 392 and 425 which are those horses showing the highest response it will be seen that they are high in both viperine and colubrine antibodies. The same applies, although with a relatively higher viperine response to horses showing the lowest titres such as Horses 266 278 371 and 395

As in the case of bacterial antitoxin such as diphtheria and tetanus obtained from horses immunized with formolised toxoid viperine and colubrine antivenene obtained from horses immunized with anavenoms do not show any sign of deficiency in neutralization nor in avidity against the respective venoms.

The different toxic and antigenic fractions demonstrable biologically and experimentally in the original venoms are found to be neutralized by the antivenene obtained from horses immunized with the respective anavenoms This applies equally well to the neurotoxin of various colubrines (*N flava N haie* etc *Sepedon haemachetes Dendraspis angusticeps*) and to the proteolytic, coagulant, anticoagulant or haemolytic fractions contained in the various African and Asiatic viperine or colubrine venoms which we have detoxicated. This maintenance in the specificity of anavenoms is particularly evident in cases of cross neutralization with monovalent antivenenes, obtained from viperine or colubrine venoms of close zoological relationship but differing in one or other antigenic fraction. Such is the case with venoms of *B arietans* and *B gabonica*, an equatorial representative of the same *Bitis* group As shown by GRASSET and ZOUTENDYK (1938) the latter venom, although closely related to that of *B arietans* is not neutralized by *B arietans* antivenene and will kill by its higher coagulation action. Specific *B gabonica* serum prepared from the respective anavenom has proved of full neutralizing and therapeutic value, not only against *B gabonica* venom but also against *B arietans* venom.

Not only the specific but also the group antigenic properties of venoms are conserved in detoxication. This is shown by the group neutralization of monovalent anti-*N flava* serum against venoms of other Naia varieties (*Naia haie N nigricolis* and other distinct species such as *Sepedon haemachetes*) The same applies to anavenoms derived from viperine venoms and demonstrated in the group neutralization of monovalent *B arietans* serum towards the venom of viperines of the same group as *B cornutus B caudalis* and other species such as *Causus rhombeatus*

As regards avidity standardization experiments on mice injected with venom-antivenene mixtures just after preparation and after 60 minutes contact,

show that maximum accuracy in titration is observed in animals injected after 1 hour contact. A great proportion of the mice injected with the neutral mixture do recover however when injected within the few minutes following the venom-antivenene mixtures.

Specific and group neutralizing antibodies contained in antivenenes prepared by means of anavenoms are concentrated by fractional salt precipitation according to the same method and with the same results as in the case with antivenene derived from horses immunized with non-modified venom (GRASSET 1932) In practice batches of 40 l. of pooled polyvalent antivenene are submitted to fractional precipitation by the successive addition of 11·5 per cent. and 6·5 per cent anhydrous sodium sulphate. The pseudo globuline so obtained contains three to four times the concentration of both viperine and colubrine antibodies. One c.c of the concentrated polyvalent antivenene is adjusted to neutralize from 12 to 15 mg B arietans and 1 to 1·5 mg N flava venoms The following figures are an example of the specific group neutralization of a batch of concentrated antivenene

1 c c of this concentrated serum has been found to neutralize —

VIPERIDAE.	COLUBRIDAE.
12 mg Bitis arietans	1 mg Naia flava.
25 mg Causus rhombeatus	1-4 mg Sepedon haemachetes.
	2 mg Naia nigricollis
	0·9 mg Dendraspis angusticeps

Comparative antivenomous response according to dosage and route of anavenom injection.

Development of viperine and colubrine immunity in horses immunized with B arietans and/or N flava anavenoms. For the purpose of this study horses submitted to the usual scheme of immunization with B arietans and/or N flava anavenoms (25 50 100 200 400 600 800, 1 200 mg) were bled 7 days after each injection. Neutralization tests were carried out in rabbits with mixtures of decreasing volumes of serum (10 to 3 c.c.) against various multiples of m.l.d. of B arietans venom.

Horse 102. 10 c.c. of serum corresponding to bleedings done 7 days after the first second and third injections of B arietans anavenom (25 mg 50 mg and 100 mg) failed to neutralize 3 mg of B arietans venom. Seven days after receiving the fourth injection of 200 mg. anavenom, 10 c.c. of the serum of this horse neutralized 3 mg of B arietans venom. After two further injections of 400 and 600 mg of the same anavenom, 7 c.c. of the serum neutralized 5 mg. of B arietans venom. After the eighth injection of 1 200 mg. of anavenom, 3 c.c. of the serum neutralized over 10 mg of venom of B arietans This titre, obtained in 8 weeks, already represents the minimum required for therapeutic purposes. A final injection of 1 400 mg anavenom brought the neutralizing titre up to 14 mg of B arietans venom.

Horses 351 and 352   In another experiment, two new horses, Horses 351 and 352, were submitted to only four large subcutaneous injections of mixed *B anetans* and *N flava* anavenom starting with 500 mg , i e a dosage twenty times higher than in the last experiment, on 22.8.39 followed by 750 mg on 14 9.39 1 000 mg on 21 9.39 and 1 400 mg on 1 10.39 of a mixture of each of these two anavenoms   Neutralization tests with samples of blood taken 7 days after the first injection showed that 10 c.c. of serum of both horses failed to neutralize either 2 mg of *B anetani* or 0 35 mg of *N flava* venoms The antivenomous immunity developed gradually during the 2nd week as shown by the titration of serum from bleedings carried out after the 20th day (11.9.39)   3 c.c. of serum from Horses 351 and 352 then neutralized respectively 3 mg *B anetans* venom. With regard to *N flava* venom, 10 c.c. of serum from horse 351 neutralized 0 35 mg   10 c.c. of serum from Horse 352 tested under the same conditions resulted in death after delay

Bleeding 23 days (14 9.39) after injection   The sera of the two horses now neutralized respectively 5 mg *B anetans* and 0 35 mg *N flava* venom.

Bleeding 7 days (21.9 39) after the second injection of 750 mg of *B anetans* and 750 mg *N flava* venoms   3 c.c. of the sera of the two horses neutralized 6 and 7 5 mg *B anetans* venom   10 c c. of the serum neutralized 0 7 and 1 mg *N flava* venom.

Bleeding taken 7 days (27 9.39) after the third injection of 1 000 mg *B anetans* and *N flava* anavenoms   3 c.c. of the serum from Horse 351 neutralized incompletely 7 5 mg. *B anetans* venom   3 c.c. of the serum from Horse 352 neutralized 8 mg *B anetans* venom   3 c.c of the serum from Horse 351 neutralized 0 5 mg *N flava* venom   3 c.c. of the serum from Horse 352 neutralized incompletely 0 5 mg *N flava* venom with death overnight

Final bleeding taken 7 days after the fourth injection of 1 400 mg anavenom 3 c.c. of the serum from these two horses neutralized respectively 8 and 10 mg *B anetans* and 0·6 and 0·65 mg *N flava* venom   These titres obtained in 44 days reflect again the considerably higher antivenene response to *B anetans* than to *N flava* venom.

From a practical point of view although the anti *B anetans* titre so reached is within close limits to the minimum required titre for therapeutic use, the antibody response remains definitely too low   These inferior results together with the big swellings which followed the original injection of a large dose of detoxicated venom protein (1 gramme of mixed anavenom for the first injection) plead for the use of progressively increasing doses of anavenoms according to the usual method described above which results in a higher neutralizing titre of both colubrine and viperine antibodies.

IMMUNIZATION OF HORSES BY INTRAVENOUS INJECTIONS OF ANAVENOMS.

We have mentioned before that large amounts of viperine and colubrine anavenoms can be injected intravenously to experimental animals without

toxic symptoms. Having in mind that the intravenous administration of anavenoms might eventually result in an increase in the immunity response, we have proceeded to the hyperimmunization of five horses on these lines. They were submitted to the usual scheme of anavenom dosage as for subcutaneous injection except that the mixed viperine and colubrine anavenom was given intravenously *N flava* anavenom used for this purpose was treated as usual with 0·8 per cent. formol but was strongly buffered in order to obtain an anavenom free from any precipitation. This antigen was used mixed in equal parts with *B arietans* anavenom (0·4 per cent. formol). In order to minimize the risk of venom proteinic shock, the mixed anavenom was added prior to injection to an equal volume of saline and injections were done very slowly

Horses 268, 392 and 395 were submitted to a course of eight intravenous injections according to the following table —

TABLE IV

Injection.	Date	*B. arietans* and *N flava* ana enom		Vol. in c.c.
No.		M		
1	15 1 41	25	25	5 + 5 saline
2	22.1 41	50	50	10 + 10 saline
3	28 1 41	100	100	20 + 20 saline
4	4—41	200	200	40 + 40 saline
5	11—41	400	400	80 + 80 saline
6	18—41	800	800	160 + 160 saline
7	25—41	1 000	1 000	200 + 200 saline
8	5.3.41	1 400	1 400	90 + 90 saline

Horses 393 and 396 received the same course of intravenous injections but carried out at intervals of 10 days. No appreciable reactions followed the first dose except a rise in temperature to 101 to 102 on the evening of the day of injection. From the 6th injection (total 1,600 mg. anavenom) onwards the horses started to show violent shock reaction. During the minutes following intravenous injection the horses became giddy or suddenly collapsed, to rise again after a few minutes of acute dyspnoea and semi unconsciousness on the ground. This was followed by shivering attacks, sweating and sharp rise of temperature to a maximum of 103 on the same evening In order to minimize the severity of these reactions, the respective doses from the fifth injection onwards were split into three fractional injections at 10 a.m., 12 a.m. and 3 p.m. and were preceded by a heart stimulant.

The adoption of this scheme tended to reduce the severity of the shock, but not inhibit it altogether The maximum reaction was usually observed

after the first fractional injection in the morning followed as a rule by collapse. Two additional injections usually resulted in a short period of apparent malaise of a much less distressing character

Preliminary neutralization tests were done on samples of blood taken a week after the seventh injection   3 c.c. of serum from the five horses failed to neutralize either 8 mg of *B arietans* or 0·8 mg of *N flava* venom   Similar tests were repeated on the 7th day after the eighth injection with the following results —

Serum—Horse 268

3 c.c. serum + 8 mg *B arietans*	Rabbit survived.
3 c.c. serum + 10 mg *B arietans*	Rabbit died in 1 hour 30 mins
3 c.c. serum + 0 8 mg *N flava*	Rabbit died in 24 hours.
3 c.c. serum + 1·0 mg *N flava*	Rabbit died in 6 hours

The sera of the other four horses 392, 395 (injections at 7-day intervals), 393 and 396 (injections at 10-day intervals) all failed to neutralize either 8 mg *B arietans* or 0 8 mg *N flava* under similar conditions. Early death occurred within 1 to 2 hours with *N flava* and within 4 to 6 hours with *B arietans* venom

These results compare unfavourably with those obtained when the same scheme and dosage of the same anavenoms are used, but injection is subcutaneous. Administration of higher doses did not appear practicable considering the distressing type of reactions observed. The poor immunity response obtained together with the risks incurred when using such a procedure did not appear to warrant further practical trial along these lines

Bitis gabonica ANAVENOM AND ITS USE IN THE PREPARATION OF A SPECIFIC ANTIVENENE.

While anti-*B arietans* serum exerted a high group neutralization against most of the South African viperines (*B cornutus B caudalis B atropos* and *Causus rhombeatus*) it afforded but a negligible protection against the venom of *B gabonica* (Gaboon viper). (GRASSET and ZOUTENDYK 1938).

This necessitated the preparation of a specific antivenene against the bite of this Equatorial African viperine. Although actually its venom is of an average toxicity the usual gravity of the bite of   gaboon adder   can be partly explained by the exceptionally large amount of venom which can be injected by this largest specimen of all African venomous snakes. From data recorded by Dr CECCALDI Director of the Pasteur Institute, Brazzaville who kindly supplied us with large supplies of this venom and from our own information, 3 5 to 5 c c of this venom can be extracted in one milking of the glands of a specimen of 1 2 m. to 1·6 m. in length. This represents a weight of 0·6 gramme to 1 gramme of dry venom (According to Dr CECCALDI s figures 2·97 grammes were obtained from three large specimens.)

F

A 1 per cent. solution of *B gabonica* venom was found to be detoxicated by 0 7 per cent. formol in Martin s broth after 4 weeks at 37° Rabbits immunized with the resulting anavenom showed a high specific immunity against *B gabonica* venom, and also group protection against *B arietans* venom. The incorporation of *B gabonica* anavenom in the hyperimmunization of polyvalent antivenene horses resulted in a serum possessing high neutralizing powers against that venom.

During the period 1933–43 seven horses were used for the preparation of polyvalent antivenene, including *B gabonica* for Equatorial Africa. In addition to the usual *B arietans-N flava* immunization they received six doses of 100 200 400 600 800 and 1 400 mg of *B gabonica* anavenom incorporated in the weekly injections. Re immunization consisted of three or four injections of 400 800 1 200 and 1 400 mg of the same anavenoms. The neutralizing titre of the bleedings of these horses so immunized, varied from 12 to 16 mg. of venom of *B gabonica* for 3 c.c. of serum. After concentration, 1 c.c. of the final therapeutic antivenene had an average neutralizing titre of 12 mg. to 14 mg *B gabonica* 12 to 14 mg *B arietans* and 1 mg *N flava* venom.

Bitis nasicornis VENOM DETOXICATION AND ANAVENOM

Experiments similar to those referred to above in connection with *B gabonica* were carried out with *B nasicornis*

Neutralization tests done with two specimens of 20 grammes of this venom obtained from Dr CECCALDI of the Pasteur Institute, Brazzaville showed only partial neutralization with polyvalent *B gabonica-B arietans-N flava* serum. 1 c.c. of this concentrated serum neutralized only 6 mg *B nasicornis* venom as compared with 12 mg *B gabonica* and 12 mg *B arietans* i e a 50 per cent. protection ratio Detoxication of *B nasicornis* venom was undertaken on the same lines as for other *Bitis* representatives. One per cent. solutions of the venom in saline and in broth were treated with 0 5 0·75 and 1 per cent. formol

Comparative atoxicity tests with these products were done after periods of 30 to 40 and 60 days incubation at 37° On the whole detoxication was found more rapid for the solutions in broth than for those in saline. The 0·75 per cent formol solution in broth was sufficiently detoxicated after 40 days incubation to be injected subcutaneously at the rate of 2 c.c. (20 mg of the original venom) to guineapigs and rabbits without production of haemorrhagic lesions. The *B nasicornis* anavenom so obtained was injected at weekly intervals in increasing doses of 10 25 50 and 50 mg to rabbits Eight days after the last injection these rabbits were tested by intravenous injections of 15 to 23 5 mg. *B nasicornis* representing 10 to 15 m.l.d. for the control rabbits and all survived 0·5 c.c. of serum from immunized rabbits neutralized *in vitro* 0·2 mg *B nasicornis* venom representing 6 m.l.d for the control rabbits

Steps were taken with a view to securing a sufficient stock of *B nasicornis*

venom to incorporate it in the preparation of polyvalent antivenene for Equatorial Africa. Immunization of two horses using this anavenom is now in progress.

Naia tripudians *anavenom* — Its *substitution for* Naia flava *anavenom in the preparation of South African antivenene*

The detoxication of *N. tripudians* venom and its transformation into ana venom has been achieved by RAMON (1925). Experiments carried out by E. GRASSET and A. ZOUTENDYK (1935a) have shown the close toxic and antigenic relationship existing between venom of the African *N. flava* and the Indian *N. tripudians* This relationship is reflected in the close cross neutralization observed in the respective antivenene towards the heterologous venom. A similar cross protection has been demonstrated in rabbits immunized with anavenoms derived from these two venoms

These findings proved of practical application in 1941 As a result of the war antivenene production by this Institute had to be considerably increased to cope with the military requirements, and supplies to various African allied authorities (French, Belgium and Egyptian) which before the war were obtaining their antivenene supplies from the Pasteur Institute, Paris. The number of horses used for the preparation of antivenene which was eight in 1938 reached twenty three in 1942.

The stock of venom of the African *N. flava* on hand became insufficient to deal with this increased production. On the grounds of the above antigenic relationship it was decided to obtain some *N. tripudians* venom from India and substitute this venom for part of the *N. flava* venom in the immunization of horses A first consignment of 100 grammes obtained in 1941 was detoxicated in Martin's broth with 0 8 per cent. formol, after the adjustment of the pH to 7 5 The detoxicated product, obtained after a month at 37° was used as anavenom for immunization purposes. A series of nine antivenene horses were re immunized with a mixture of antigen consisting of 50 per cent. *N. flava* and 50 per cent. *N. tripudians* anavenom. Ten other antivenene horses received exclusively *N. tripudians* anavenom mixed with *B. arietans* anavenom as in the first series Some of these horses were tested at the end of the two subsequent immunizations against the same doses of *N. flava* venom as in the past. The results of this experiment showed that not only did the anti *N. flava* neutralizing titre of the horses remain the same but the titre of some immunized with *N. tripudians* increased as the result of its substitution in place of the *N. flava* Further supplies of *N. tripudians* venom were obtained and detoxicated accordingly From June, 1941 until the middle of 1943 more than 200 grammes of venom of V *tripudians* were detoxicated and the resulting anavenom was used in equal parts with *N. flava* venom for six subsequent re-immunizations of twenty horses in antivenene production. These courses entailed 3 weekly injections as indicated in the following table

TABLE V

Injection No.	N. *tripudians* in mg.	N. *flava* in mg.	B. *arietans* in mg.
1	200	200	400 + tapioca
2	400	400	800 + tapioca
3	800	800	1 600 + tapioca

A sample was taken for preliminary titration after 8 days.

9th day bleeding—10 1

11th day bleeding—10 1

The anti-N *flava* titration carried out on the respective bleedings of these successive re immunizations showed that on the whole either the N *flava* neutralizing titre was maintained or an increase in the titre up to 40 per cent. in some horses took place. The resulting concentrated antivenene showed the usual three to four fold concentration ratio in N *flava* antibodies, as is the case in pure anti N *flava* concentrated globulin.

Group neutralization against other African colubrine venoms such as N *haie* N *nigricollis* Sepedon *hæmachates* and Dendraspis *angusticeps* was also ascertained and found to be the same as in the case of the concentrated anti-venene derived from only N *flava* venom. Besides this useful application of N *tripudians* anavenom to the present purpose, these results provide us with further information regarding the application of anavenoms, i.e., the production of specific anti-N *tripudians* antivenene by means of its anavenom.

While on the subject of detoxication of venoms from snakes of Asiatic origin, we shall refer to experiments regarding the detoxication of two other Indian representatives and the use of their specific anavenoms in respective immunizations

Bungarus fasciatus (Banded Krait). The venom of this Indian colubrine is characterized, in addition to its neurotoxin by a higher content of haemorrhagin than is found in N *tripudians* venom. The specimen of *Bungarus fasciatus* venom we used was supplied by the Pure Drug House Calcutta. Detoxication was carried out on the same lines as for Asian venoms. Saline and Martin a broth were both used as solvent mediums for detoxication and the results compared. The latter as shown earlier in this paper considerably accelerates detoxication and lowers the percentage of formol necessary for the production of anavenom.

(1) 1 per cent. saline solution of B *fasciatus* was treated respectively with 0 8 1·0 and 1 5 per cent. of 40 per cent. formol. (2) 1 per cent. solutions of the same venom in Martin a broth were treated respectively with 0·6, 0·8 and 1·0 per cent. of formol.

Tests for detoxication were carried out after a period of 30 days at 37° on the various samples. Amounts of 1 and 2 c.c. for each sample (10 and 20 mg of the original venom) were injected subcutaneously into normal guineapigs. Slight local necrosis was observed in the animals injected with 1 per cent. and 1·5 per cent. formol saline solution and was accompanied by a soft oedema of the abdomen in the animals treated with 0·8 per cent. formol. The animals injected with either 0·6 0·8 or 1 per cent. formol solution in broth showed no necrosis nor any appreciable swelling at the site of injection. It appears, therefore that 0·6 per cent. formol is sufficient for detoxication of the *B. fasciatus* venom in 30 days at 37° provided broth is used as the medium of detoxication   In order to test the antigenicity of the detoxicated products three rabbits of 2,000 grammes were submitted to a series of five subcutaneous injections of 10 20 40 60 and 80 mg of broth detoxicated *B. fasciatus* anavenom on 2.9 40 9.9 40 16.9 40 26.9 40 and 3 10.40   Ten days after the fifth injection, the three rabbits together with the controls were submitted to an intravenous injection of a multiple 3 to 15 lethal dose of *B. fasciatus* venom.

Rabbit 1   Tested with 3·6 mg (3 m.l.d.) *B. fasciatus*   Survived. Reinjected after 20 hours with 3·6 mg (3 m.l.d.)   Survived. (Altogether 6 m.l.d.)

Rabbit 2.   Tested with 6 mg (5 m.l.d.) *B. fasciatus*   Died after 14 hours

Rabbit 3   Tested with 6 mg (5 m.l.d.) *B. fasciatus*   Survived Reinjected after 36 hours with 12 mg (10 m.l.d.)   Survived. (Altogether 15 m.l.d.)

Control Rabbit 1   Tested with 1 mg *B. fasciatus*   Died after 8 to 14 hours.

Control Rabbit 2.   Tested with 1 2 mg *B. fasciatus*   Died after 2 hours

Control Rabbit 3   Tested with 15·0 mg *B. fasciatus* Died after 15 mins From these experiments it appears that a specific anavenom can be obtained from *B. fasciatus* venom which is capable of imparting to immunised animals an active immunity against a high number of lethal doses of that venom.

Vipera russellii (Daboia)   Let us finally refer briefly to the detoxication of the Indian *Vipera russellii* (Daboia) which was achieved in 3 weeks by treatment with 1 per cent. formol (GRASSET and ZOUTENDYK, 1935a)   Active immunity was obtained in rabbits submitted to a series of five injections of a total of 500 mg Daboia anavenom.   One c.c. of the serum neutralized *in vitro* up to 1 mg Daboia venom (5 m.l.d. intravenously for control rabbits)   Other rabbits which received only three doses of 30 40 and 50 mg *B. russellii* anavenom stood the test of 4 mg of the same venom injected intravenously *i.e.* 20 m.l.d. for the controls.

Sepedon haemachetes (Ringhals) anavenom
Preparation of specific polyvalent antivenene

As a matter of immunological interest, we shall refer to certain investigations undertaken with the venom of *Sepedon haemachetes* and its anavenom, in connection with the production of antivenene

As shown by E. GRASSET and ZOUTENDYK (1933) the venom of this South African colubrine can be detoxicated on the same lines as the venoms of the *Naja* group. A 1 per cent. solution of *S. haemachates* dissolved in Martin's broth and treated with 0.8 per cent. formol was rendered atoxic after 1 month of incubation at 37°. This anavenom was used for the active immunization of experimental animals and for the hyperimmunization of horses. Cross neutralization tests which were carried out using *S. haemachates* and *N. flava* sera (obtained from horses immunized with the respective anavenoms) monovalent towards the respective venoms, showed that *S. haemachates* possesses consider ably lower antigenic powers as well as toxicity as compared with *N. flava* venom. (For rabbits 2,000 grammes m.l.d. *N. flava* = 0.25 mg. and *S. haemachates* = 0.95 mg.)

The following example serves to illustrate these facts.

Horse 349 was immunized with the following course of *S. haemachates* anavenom injected at weekly intervals.

TABLE VI

Injection No.	Date.	*S. haemachates* anavenom in c.c.
1	6.10.40	50
2	13.10.40	100
3	20.10.40	200
4	27.10.40	400
5	3.11.40	800
6	10.11.40	1,200
7	30.11.40	1,800

Three c.c. of the serum from the bleeding 7 days after the seventh injection of anavenom neutralized over 1 mg of *S. haemachates* venom but less than 1.2 mg. On the other hand, the same amount of serum failed to neutralize 0.8 mg of *N. flava* venom (death took place in 1 hour 30 minutes). the neutralizing limit was about 0.6 mg.

Similar cross neutralization tests carried out with a monovalent anti-*N. flava* serum against *S. haemachates* venom, showed by contrast that the group protection against the latter venom is considerably higher than that observed with anti-*S. haemachates* serum of equivalent specific titre against *N. flava* venom.

3 c.c. *N. flava* serum + 1 mg *N. flava* venom. Rabbit survived.

3 c.c. *N. flava* serum + 1 mg. *S. haemachates* venom. Rabbit survived. This is the reason why although *S. haemachates* venom is much more easily obtained than *N. flava* the latter venom was substituted for the former in 1932 as the group antigen in the preparation of polyvalent South African antivenene.

Detoxication of Opistoglyphe colubrine venoms
Dispholidus typus anavenom

Studies on this group of venoms deserve special attention. Investigations on *Dispholidus typus* (Boomslang) venom (GRASSET and SCHAAFSMA 1940) gave evidence of the wide difference in toxicity and antigenic constitution of the African Opistoglyphe and Protoglyphe colubrine venoms. Besides a neurotoxic fraction this venom is characterized by the presence of an extremely powerful coagulant principle *in vivo* and *in vitro* (the intravenous m.l.d. for the pigeon is 0·0003 mg). It is similar in its action to the high coagulant powers exerted by the venom of the Australian Elapina *Notechis scutatus* (Tiger snake). A very limited neutralization, however, is exerted by anti-*N. scutatus* serum against *D. typus* venom. Attempts to neutralize this venom with other types of antivenenes such as polyvalent *N. flava-B. arietans-B. gabonica* anti-*N. tripudians-Vipera russellii* serum or the mixture of other antineurotoxic and anticoagulant antivenenes have not proved successful.

These results however have been easily reached by the preparation of a specific antivenene. Detoxication of *D. typus* venom was realized by the treatment of a 1 per cent. solution in saline with 0·8 per cent. formol after 4 weeks incubation the resulting product being devoid of coagulating powers both *in vivo* and *in vitro*. The serum of animals immunized with increasing doses of the specific anavenom possessed high neutralizing anticoagulant powers against *D. typus* venom. 0·25 c.c. serum neutralized 0·006 mg of this venom, i.e. over 20 m.l.d. of *D. typus* venom for the pigeon.

CONCLUSIONS AND SUMMARY

By submitting viperine and colubrine venoms to formol detoxication under optimum conditions, atoxic, antigenic derivatives or anavenoms of the following venoms were obtained.

VIPERIDAE.

African Viperidae *Bitis arietans B. caudalis B. atropos B. gabonica B. nasicornis* and *Causus rhombeatus*

Asiatic Viperidae *Vipera russellii* (Daboia)

COLUBRIDAE.

African Proteroglyphe *Naia flava N. haia N. nigricollis Sepedon haemachetes* and *Dendraspis angusticeps*

African Opistoglyphe *Dispholidus typus*

Asiatic Proteroglyphe *Bungarus fasciatus*

The optimum conditions for the transformation of venoms into anavenoms are controlled by a number of factors which must be taken into account for each venom —

(1) Concentration of the original venom solution.

(2) Biochemical constitution of the solvent used as the medium of detoxication.

(3) Concentration of formol.

(4) pH and buffer

(5) Temperature.

(6) Period of detoxication.

The preparation and control of anavenoms are described. The following method is applied to the hyperimmunization and re-immunization of horses for the production of antivenene By means of six to eight weekly injections of increasing doses of 1 per cent. specific anavenoms, therapeutic antivenenes have been obtained within 2 to 3 months against the following venoms.

Colubridae   anti *N flava* serum   anti-*S haemachetes*   anti-*N tripudians*

Viperidae   anti-*B arietans* serum   anti *B gabonica.*

By mixture of the respective anavenoms in adequate proportions a polyvalent antivenene has been prepared against the above-mentioned African snakes.

The preparation is given of a polyvalent *B arietans*-*N flava* antivenene with reference to anti viperine and colubrine neutralizing titres of forty-six horses immunized by this method during the period 1933–43

Reference is made to the polyvalence and group neutralization of the natural and concentrated antivenene also to the suitable substitution of the Indian *N flava* anavenom for *N flava* anavenom in the preparation of this serum based on the close antigenic relationship of these two colubrine venoms.

The advantages resulting from the use of anavenoms, as compared with non-modified venoms in the preparation of antivenenes are discussed.

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STUDIES IN LEISHMANIASIS IN THE
ANGLO EGYPTIAN SUDAN

VII ESPUNDIA IN A MONKEY INFECTED EXPERIMENTALLY
WITH SUDAN KALA-AZAR*

BY

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KIRK and DREW (1938) pointed out that the three classical forms of leishmaniasis—visceral, oral and cutaneous—occur in the endemic areas of the Sudan. The relationship between the three types of infection has been discussed in two previous papers in this series It was stated that any suggestions made in these papers were based on the observation of naturally contracted infections in the human subject, since, with the facilities at our disposal, attempts to differentiate strains of leishmania by animal inoculation were so variable and included so many failures to produce any type of infection that no useful information regarding differentiation of strains was obtained by this method.

The observations recorded in the present communication, being merely part of the wider investigation mentioned above do not in any way serve to clarify matters, but they are not inconsistent with the suggestions made in previous papers, and seem to us of sufficient interest to be recorded separately Briefly the purpose of the present article is to record the development of an espundia like lesion in a monkey inoculated intraperitoneally with material from a case of Sudan visceral leishmaniasis in the third passage in monkeys

HISTORY OF THE STRAIN

The strain was originally obtained in August, 1941 from a Sudanese soldier a case of kala azar in the River Military Hospital, Khartoum. Splenic puncture was done, and the material thus obtained inoculated directly into the peritoneal cavity of a monkey (*Cercopithecus aethops* L.). This animal developed visceral leishmaniasis, which ended fatally but during the course of the infection material from a splenic puncture was inoculated intraperitoneally into another monkey of the same species. This second monkey also developed visceral leishmaniasis, but in this case the infection appears to have ended in spontaneous recovery This second monkey was subjected to splenic puncture on numerous occasions during the course of its illness, on two of which (7 10 42 and 1 11 42) material thus obtained was inoculated intraperitoneally into a third monkey of the same species. During the following 10 months this third monkey remained in good condition, and there was no enlargement of liver or spleen. It was considered likely that the passage had been a failure and that the strain had been lost.

PRESENT CONDITION

About the middle of January 1944 it was noticed that the monkey had a small sore on its nose, but no great attention was paid to this, nor was the monkey examined closely During the next 5 weeks the sore continued to enlarge under a scab The animal was examined in the last week of February 1944 Removal of the scabs revealed an oro-nasal lesion of the character and dimensions shown in the photograph. Typical leishmania were readily found in smears from the exudate, and also in smears made from a piece of tissue excised from the outer and lower edge of the sore. Two discrete rupia-like scabs were seen on the animal's tail removal of these revealed shallow indolent, circular ulcers about ¼ inch in diameter and smears made from the ulcers contained numerous leishmania. There was also a small ulcerative lesion on the middle finger of the right hand, in which leishmania were found. Examination of the abdomen revealed considerable enlargement of the spleen and material from a splenic puncture contained leishmania. There was also noticeable a marked deterioration in the animal's general condition.

Comments

One or two points require some further comment.

1 Khartoum lies outside the endemic area of leishmaniasis in the Sudan. During the half century which has elapsed since the inauguration of the present administration in 1898, Khartoum, being the capital, has been under closer medical supervision than any other place in the country Many imported cases of leishmaniasis have been treated in the main hospitals there, but, with one doubtful exception, no autochthonous cases have been reported in Khartoum.

Therefore it seems safe to assume that coincidental infection with oriental sore or any other strain of leishmaniasis likely to cause oro-nasal lesions can be excluded in the present instance, and that the oro-nasal lesion which developed in this monkey was caused by the parasites which were inoculated intraperitoneally from the splenic pulp of the previous monkey

2. Past experience in the laboratories in Khartoum provides no evidence that *Cercopithecus aethiops* has any special tendency to develop oro-nasal lesions when infected with Sudan visceral leishmaniasis although the animal can be

ESPUNDIA
IN A MONKEY
INFECTED
EXPERIMENTALLY
WITH SUDAN
KALA AZAR.

infected readily by the nasal route. (ARCHIBALD and MANSOUR 1937 KIRK, 1942) and parasites can often be found in nasal smears from animals suffering from kala azar Among the numerous monkeys infected by MARSHALL (1911 1913) ARCHIBALD and MANSOUR (1937) HORGAN (1944) the present writer and other workers in the Sudan, this is the only one in which an oro-nasal lesion of the espundia type has been observed. Although the parasites of Sudan kala-azar sometimes cause oro-nasal lesions, this probably happens only in occasional cases and it may be noted that in the present instance the oro-nasal lesion appears to be part of a fairly widespread cutaneous infection. We have previously suggested that the condition is somewhat similar in the case of naturally contracted infections in the human subject.

3. In previous papers it has been suggested that certain strains of leishmaniasis may have a greater tendency than others to the production of oro-nasal conditions. Unfortunately in the present instance the data available are insufficient to indicate whether the strain had any special tendency to produce oro-nasal lesions in the human subject. The patient from whom it was obtained was seen by the writer on one occasion and for a few moments only. He was a fairly average case of kala-azar with the usual signs of fever splenomegaly and leucopaemia. There were no obvious cutaneous or muco-cutaneous manifestations, but the patient was not subjected to the detailed clinical examination used by KIRK and SATI (1940) in this connection. Treatment was started immediately after the inoculation of the monkey and the patient made a good recovery. As far as we know he is still well.

NOTE ON THE MONKEY

The monkey here called *Cercopithecus aethiops* L. is the common grivet monkey of the Sudan. There is much confusion in the literature relating to the nomenclature of this species. ELLIOT (1912) attributes the confusion to LINNAEUS himself inasmuch as LINNAEUS apparently created the species (*Simia aethiops*) without depositing a type specimen, and perhaps without even seeing it.

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AN INVESTIGATION INTO THE NUMBER OF SPOROZOITES FOUND IN THE SALIVARY GLANDS OF ANOPHELES MOSQUITOES

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In 1937 I described a technique for injecting known numbers of sporozoites (SHUTE, 1937) Since then the technique has been improved and the time required in actual counting considerably reduced; and it is believed, a greater degree of accuracy is now achieved. It is therefore proposed to describe the improved method and to discuss the results obtained

Ross (1910) in his book *The Prevention of Malaria* discussing the average number of sporozoites (protospores) in a mature oöcyst (zygote) estimated the actual number at about 1 000 He considered it unlikely that more than 10 000 sporozoites would be found in the glands of a single anopheline. Discussing the number of sporozoites which enter the blood during the act of biting Ross states   this must depend (a) upon the number of spores in the insect s salivary glands and (b) upon the number of times the insect bites its victim. He continues,   because mosquitoes inject their poison before commencing to suck blood, an insect which bites a person several times (as, for instance, when he is asleep), is likely to inoculate many more sporozoites than one which succeeds in biting only once." In the former case several thousand sporozoites may perhaps be introduced in the latter case perhaps only a few Ross concludes that not all of the spores which are injected by the mosquito are likely to live. Probably many perish by falling outside the blood stream or by becoming a prey to phagocytes

On page 87 of his book, Ross states   that the largest number of oöcysts found by him on a single mosquito s gut was 445 (*Culex fatigans* and *Proteosoma*)   If therefore, all these oöcysts reached maturity and ruptured normally and if subsequently all the sporozoites succeeded in finding harbour in the salivary glands then according to Ross s estimate that an oöcyst produced about 1 000 sporozoites the number developing from 445 oöcysts would be about 445 000   Therefore about 2 per cent of sporozoites from oöcysts succeed in reaching the salivary glands and if as Ross suggests, an oöcyst produces about a thousand sporozoites only about twenty from each oöcyst ever reach the glands

As the result of a simple technique (described at the end of this paper) whereby the number of sporozoites in the glands of a mosquito can be estimated, some recent experiments indicate that the number able to harbour in the salivary glands of an anopheline mosquito greatly exceeds the 10 000 suggested by Ross

The following experiments were undertaken to determine the maximum number of sporozoites in the glands and also how many blood meals are necessary before all, or nearly all, of the sporozoites are discharged.

EXPERIMENT 1

Species of parasites—*P falciparum* Strain Rumanian
Species of mosquito—*Anopheles maculipennis* var *atroparvus*
Temperature of Insectary—75 to 80 F

In this experiment a batch of mosquitoes was given two infected feeds on alternate days. After sporozoites appeared in the salivary glands the mosquitoes were given a non infective blood meal every other day and at each multiple of 6 days three mosquitoes were dissected an emulsion made of the glands of the three mosquitoes and the sporozoites counted. Negative glands encountered during the dissections were discarded. Only complete sets of glands, all six lobes, were used.

During the odcyst development stage and before any had ruptured, forty three mosquitoes were dissected, and of these 84 per cent. were positive. Seventeen had at least 100 odcysts, fourteen 50 to 100 and five had 50 or less.

Odcysts began to rupturing on the 15th day after the first infected feed, and sporozoites were found in the salivary glands in small numbers the following day. Two days after gland infection, a number of mosquitoes was dissected and none of the stomachs showed odcysts and thus it was shown that the infection had passed on to the glands.

Day after sporozoites in gland	Number of feeds on rabbits blood.	Sets of glands.	Number of sporozoites.
	1	3	64,200
15	6	3	78 000
3	1	3	61 50
41	1	3	34,500

Following the twentieth feed, 44 days after the sporozoites had appeared in the glands, only seven mosquitoes remained alive. These were dissected and the sporozoites of each mosquito counted separately. Two were negative the five positive mosquitoes showed 17 100 5 700 3 400 950 and 30 sporozoites respectively

From this experiment it will be seen that there was no appreciable decrease in the numbers of sporozoites until after the twenty-fifth feed.

EXPERIMENT 2.

Species of parasite—*P falciparum* Strain Rumanian.
Species of mosquito—*Anopheles maculipennis* var *atroparvus*
Temperature in Insectary—75 to 80 F

This batch was fed on two occasions on alternate days

During the oöcyst development stage the stomachs of twenty mosquitoes were dissected and of these 90 per cent. were positive The number of oöcysts per gut varied from nil to several hundreds

Oöcysts began rupturing on the 10th day after the first infected feed and sporozoites first appeared in the glands on the 11th day

Days after sporo-zoites in glands.	Number of feeds on rabbits blood.	Sets of glands.	Number of sporozoites.
8	2	1	209 000
		1	85 500
		1	66,500
		1	61 750
		1	57 000
		1	54,500
		1	33 000
		1	28 500
		1	16 600
		1	9 500
		1	950
25	8	1	1 350
44	14	1	750
		1	1 580
		2	20
50	19	1	1,000
		1	190
		1	6

EXPERIMENT 3

Species of parasite—*P falciparum* Strain Rumanian.
Species of mosquito—*Anopheles maculipennis* var *atroparvus*
Temperature in Insectary—75 to 80 F

In this experiment the mosquitoes were given only one infected blood meal. The gametocytes in the blood of the patient were very numerous over 5 000 per c mm. of blood.

During the oöcyst development stage and before any had ruptured fifty three were dissected, and of these 73 per cent. were positive. Nine had 1,000 or more oöcysts, eight 500 to 800 sixteen 100 to 500 five 25 to 50 and two had 5 to 25

Oöcysts began rupturing on the 10th day after the first infected feed, and sporozoites were found in the salivary glands on the 11th day

On the 4th day following invasion of the glands fifty mosquitoes were dissected, and of these forty four were positive and counts were made in thirty two of them.

Number of sporozoites per mosquito

219 450	95 000	86 450	85,500	82,650
82,650	76 000	72,200		
71,250	68,500	62,700	58,900	58 900
57 000	51,300	49 400		
47,500	43 700	39 800	39 000	34,200
33,250	32,300	30 400		
27 750	27 750	27 750	20 000	19 000
17 100	16 150	11 450		

The above findings represent the maximum number of sporozoites in the glands of this batch. The remainder was fed on normal blood every 3rd day

Days after sporo-zoites in glands.	Number of feeds on rabbits blood	Sets of glands.	Number of sporozoites.
2	3	1	8,500
		1	11,450
		1	8 750
12	6	1	12,480
		1	18,800
		1	6,290
*7	9	1	4,780
		1	6 700
		1	7 800
26	1	1	11 800
		1	8 500
		1	*0
45	15	1	730
		1	15
		1	5

After the seventeenth blood meal (51 days after the glands first became infected) only five mosquitoes remained alive. Four were negative and one contained fewer than twenty sporozoites.

Although the latter batch was given only one infective feed, many of the mosquitoes stomachs contained over 1 000 oöcysts and one set of salivary glands contained over 200 000 sporozoites It will be seen that after the seventeenth blood meal most of the mosquitoes had discharged all their sporozoites over a period of 50 days. There is also the possibility that many sporozoites were discharged into the vessel of water kept permanently in the cages to enable the mosquitoes to deposit their eggs Mosquitoes frequently take up water while resting on the surface during egg laying and in this way a number of sporozoites must be discharged into the water

The above experiments show that *Anopheles maculipennis* are capable of harbouring very large numbers of sporozoites in their salivary glands and that even in very heavily infected insects most of the sporozoites are discharged after about twenty blood meals. Sixty thousand sporozoites in the glands of a mosquito are frequently encountered. If as seems unlikely, the numbers of sporozoites discharged at each feeding are about equal, then a mosquito whose glands contained 60 000 sporozoites and which survived long enough (about 2 months) to feed on twenty separate occasions, would inject about 3 000 sporozoites at each biting However in very heavily infected glands it is seen that enormous numbers of sporozoites are situated in the salivary *duct* and it is presumably the case that these are mostly discharged during the first few feeds Therefore a patient who is bitten within a few days of the glands being invaded by sporozoites will receive a much heavier infection than will a patient who is bitten after the insect has fed on a number of occasions, irrespective of the number of sporozoites in the gland cells because after the insect has fed several times the sporozoites free in the duct will have been discharged, probably many thousands at each time of feeding

TECHNIQUE EMPLOYED

Apparatus used.

1   Ordinary glass slides each with a half-inch square cover-slip fixed to the centre of the slide by Canada balsam.
2.   Fine capillary tubes marked to 0·05 c.c.
3   Locke s fluid.
4   Tuberculin syringe graduated to 1 c.c.
5   Three-quarter inch square cover-slips.

The mosquito to be dissected is transferred to a narrow test tube of ⅜ inch diameter and when the insect is at the bottom of the tube rap the tube containing the insect sharply five or six times against the palm of the hand. This is sufficient to stun the mosquito It is then placed on a slide and the head is cut off cleanly with a sharp needle. A small bead of normal saline is placed on a glass slide close to but not touching the thorax The point of a dissecting needle held in the left hand is gently but firmly planted on the thorax, just below the part where the glands lie. With a needle in the right hand, gentle pressure is put on the upper part of the thorax, a little above the left hand needle This pressure is quite sufficient to cause the complete glands to protrude from the thorax and when this occurs the right-hand needle is used to lift them away from the muscular tissue which has been removed during the dissection and they are then brought into contact with the bead of saline This prevents the glands sticking to the slide. The dissected salivary glands

are then examined under the low power of the microscope to ascertain that all the lobes are present. A ¼ inch square cover-slip is then dropped on to the glands at an angle so that one corner of the cover-slip is protruding over the slide. The specimen is then examined under the 1/6th inch lens with reduced light, and if sporozoites are seen the slide is removed from the microscope to the laboratory bench, preferably on a dark surface (A piece of circular paper painted black and held in position beneath a piece of plate glass is best.) Gently but firmly pressure is applied on the cover-slip to crush the glands. Holding the corner of the cover-slip which is protruding beyond the slide, lift it without dragging this will leave the bead of fluid containing the crushed glands at the top point of the cover-slip, but on the slide With a fine pipette add two or three extra drops of sterile Locke s fluid, taking care to avoid spreading the fluid over too much of the slide Draw the fluid into the syringe and add a few extra drops of Locke s on to the slide and draw this up also If only one or two sets of glands are to be injected, several washings can be made but if many glands are to be injected the quantity of fluid for each gland should be as small as possible With care fifteen to twenty glands can be emulsed and collected in 1 c.c. of fluid

The contents of the syringe are then discharged into a small flat-bottomed watch glass and stirred thoroughly by drawing it in and out of the syringe three or four times. Prick the pad of the middle finger of the left hand and with the point of the needle lift off a speck of blood and add to the fluid this gives a suitable background on which to focus when counting sporozoites, but only a few hundred red cells are required for the purpose.

Draw up 0·45 c c of the fluid and blow it on to the square fixed cover-slip without spilling any over the slide. With a needle distribute the fluid evenly over the cover-slip and cover with a petri dish until it is quite dry Fix with methyl alcohol for a few minutes, dry and stain for half an hour with weak Giemsa stain (one part of stain to 30 parts of distilled water)

To count the sporozoites

An Ehrlich eye-piece, or its equivalent, and a 1/12th oil immersion lens are essential. Focus on to the specimen (the scanty red cells present will be helpful here), and work along until the extreme right or left edge of the cover-slip is reached Count the number of sporozoites in a horizontal strip and repeat twice at different parts of the cover-slip, and then take an average I prefer counting one strip across the top of the cover-slip, one across the bottom and one across the centre. The number of microscope fields from top to bottom of the cover slip is predetermined by ensuring a drop of blood over a cover-slip of the size to be used, and staining it in the ordinary way the corpuscles enabling one to estimate the exact number of fields. It is a good plan to do all counts with the draw tube of the microscope closed.

When the average number of sporozoites per strip is known, the total number of sporozoites in the fluid is easily and accurately calculated. If, for example there are 100 microscope fields from top to bottom of the cover slip and if the average number of sporozoites per horizontal strip is 100 then the number of sporozoites on the whole of the cover-slip is 100 × 100 = 10 000 If the quantity of fluid on the cover-slip represents 1 20th of the whole, then the total number of sporozoites equals 200,000 If, then, the whole of the fluid remaining in the syringe is injected into a patient, the total number of sporozoites injected will be 200 000 minus the 10 000 in the 0·05c.c. used on the cover slip

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Transactions of the Royal Society of
Tropical Medicine and Hygiene.
Vol XXXVIII No 6 July 1945

THE ANOPHELINE MOSQUITOES OF MELANESIA

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Since an article on the malaria mosquito of Melanesia in a previous paper
(LEVER 1942) further work has been done in the Pacific on this insect primarily
as a result of the sickness it caused during the recent campaigns in the Solomons
Papua and New Guinea. In view of its importance these further notes seem
desirable.

Systematics

A more recent publication by TAYLOR (1943a) shows that the typical
form of *Anopheles punctulatus* Dönitz is often less abundant than its variety
moluccensis Swell. and Swell., de Graaf † Through the courtesy of the Director
of the Imperial Institute of Entomology Dr S A. NEAVE, the specimens in
the National Collection were recently re-examined for the writer both varieties
being present in material from New Guinea, New Britain Solomons and New

Hebrides, while *typicus* alone was represented from the Admiralty Islands (Manus) and *moluccensis* only from Santa Cruz Islands, its eastern limit in the Solomon Islands archipelago.

Although the definite separation of the variety from the typical form was made as long ago as 1932, it appeared in German under a title suggesting it dealt only with the mosquitoes of the Netherlands East Indies (SWELLENGREBEL AND RODENWALDT 1932) and so escaped wider recognition among Pacific workers.

Habits

A useful paper is one by HEYDON (1923) who however stated that *Anopheles punctulatus* does not breed in empty tins around Rabaul New Britain, though these and other receptacles have been shown to be chosen in the Solomons (LEVER, 1933) especially in the wet season

TAYLOR (1943a) shows that *moluccensis* throughout its range will follow man into places where jungle has been felled, thereby allowing penetration of sunlight on to ground water even if brackish, which was previously unsuitable for oviposition when the bush or scrub was standing. Streams, pools, swamps and other permanent water places are the chief sites. It is easy to see what an important bearing this unfortunate predilection has on troops forced to make some kind of clearing in the jungle for bivouacking or other purposes. The scarcer variety *typicus* prefers more temporary sites than *moluccensis* i.e. wheel tracks (increased enormously due to mechanical vehicles), hoof marks, and small non brackish puddles, but curiously enough it is *molucensis* only which is recorded from receptacles such as tins and beached canoes, which *typicus* avoids.

So far as biting is concerned TAYLOR (1943a) records that in New Britain it feeds freely indoors from the mid afternoon until late at night and will feed at any time of the day if the sky be overcast and this was shown to be so by the present writer in the Solomons (LEVER, 1933) biting being reported at 11 a.m. in coconut plantations on Guadalcanal as well as indoors at 3.30 p.m. It is unwise, therefore, to restrict to the late evening precautions against being bitten and relax them in the morning say when shaving. In the same paper the present writer referred to its habits of settling on warm cups and dishes— an observation which does not seem to have been reported elsewhere. It would be interesting to know if the primary attraction to a host is due to appreciation of its body heat at some distance.

Distribution

TAYLOR (1943a) corrects his previous record (TAYLOR, 1934) of this mosquito occurring in New Caledonia, an error which has caused authors even as late as 1943 (McNULL and POPE) to include this island when giving the distribution of *A punctulatus*

TAYLOR s remark (1943b) that it seems " somewhat doubtful   if *Anopheles* may be introduced east of the 170° (eastern) meridian is not likely to be shared by those living in that area nor is his suggestion that any introduced *Anopheles* would not survive because of the fish, *Gambusia*   Residents in Fiji Samoa and Tonga know that there are many areas of brackish and other water where this useful fish does not occur and in any case TAYLOR s belief is at variance with BUXTON (1927) who states that   *Anopheles punctulatus* is not a specialist in its breeding places and it would easily establish itself in Fiji or Samoa,   and this belief is shared by others in Melanesia, including the writer who sent samples of Fiji water to the New Hebrides where oviposition and development of *Anopheles* was proved by a research worker of the United States Naval Reserve (LEVER, 1943)   TAYLOR (1943a) in correcting MUMFORD states that the Santa Cruz Islands lie   somewhat north of the Solomon Islands   but this is incorrect as they are situated some 200 miles east of the eastern Solomons and south-east, not north, of the centre of that group

Finally the fourth edition of SVENSSON s useful little book (1942) says malaria —and therefore *Anopheles*— does not occur east of meridian 160°   and also omits the Solomons and New Hebrides as islands where both occur   If this were correct the eastern half of Guadalcanal besides Malaita San Cristobal, Rennell and the Santa Cruz Islands (apart from the New Hebrides) would all be malaria free which, of course, is untrue   The correct meridian is 170 E.

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